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## **REMARKS**

Claims 1-20 are pending in the instant application. The rejections set forth in the Office Action are traversed by argument below.

### **1. Rejection of claims 1 and 2 under 35 U.S.C. § 102**

The Office Action asserts a rejection of claims 1-20 under 35 U.S.C. § 102(e), as being anticipated by U.S. Patent No. 6,433,145 (the '145 Patent). The Action accurately states that the '145 Patent discloses an amino acid sequence (*i.e.*, the amino acid sequence set forth in SEQ ID NO: 2 of the '145 Patent) that is identical to the amino acid sequence set forth of SEQ ID NO: 5 of the instant application. The Action also states that the '145 Patent discloses variants; pharmaceutically acceptable carriers; the fragment set forth in SEQ ID NO: 6 of the instant application; derivatives, including polymers; fusion proteins; and expression in eukaryotic and prokaryotic cells.

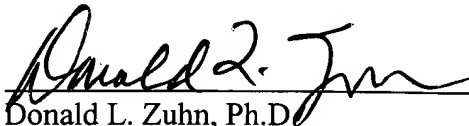
Applicants submit a Declaration under 37 C.F.R. § 1.131 establishing conception of the subject matter of the claims rejected under 35 U.S.C. § 102(e) prior to the effective date of the reference on which the rejection is based, as well as establishing that the subject matter of the rejected claims was diligently reduced to practice. Applicants note that due to the unavailability of some of the named inventors, Applicants' representative was unable to secure an executed Declaration. However, Applicants' representative will secure and promptly submit an executed Declaration containing the signatures of all three named inventors. Applicants contend that because the Declaration sufficiently establishes the conception of the subject matter of the claims prior to the effective date of the '145 Patent, and further, establishes that the subject matter of the rejected claims was diligently reduced to practice, the Declaration is sufficient to overcome the rejection of claims 1-20 under 35 U.S.C. § 102(e) as being anticipated by the '145 Patent. Applicants, therefore, respectfully request that this ground of rejection be withdrawn.

## **CONCLUSIONS**

If Examiner Andres believes it to be helpful, she is invited to contact the undersigned representative by telephone at 312-913-0001.

Respectfully submitted,  
**McDonnell Boehnen Hulbert & Berghoff**

Dated: June 10, 2004

By:   
Donald L. Zuhn, Ph.D.  
Reg. No. 48,710



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE  
(Case No. 99-372-F)

PATENT

In re Application of: Welcher et al. )

Serial No.: 09/927,850 )

Filed: August 10, 2001 )

For: Interferon-Like Molecules )  
and Uses Thereof )

Before the Examiner: J. Andres

Group Art Unit: 1646

Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

Sir:

**DECLARATION PURSUANT TO 37 C.F.R § 1.131**

We, Andrew A. Welcher, residing at 1175 Church Street, Ventura, California; Duanzhi Wen, residing at 3885 Campus Drive, Thousand Oaks, California; and Michael Kelly, residing at 790 San Doval Place, Thousand Oaks, California; hereby declare:

1. We are named co-inventors on United States Application No. 09/927,850, filed on August 10, 2001.

2. The invention disclosed and claimed in the instant patent application was conceived in the United States by us before July 21, 1998 and was then diligently reduced to practice.

3. Accompanying this Declaration are photocopies of forty-one (41) pages from our laboratory notebook showing conception of our invention before July 21, 1998. Specifically, the photocopies of our laboratory notebook show that a genomic cloning approach was used to identify the nucleic acid sequence of human interferon-like polypeptide (*see* page 34 of laboratory notebook).

Three genomic clones were identified as containing nucleic acid sequences encoding at least a portion of human interferon-like polypeptide (*i.e.*, clones 2, 6, and 7; *see* page 40). The nucleic acid sequences from these clones were isolated and then re-cloned into a suitable sequencing vector. One of the three genomic clones was determined to contain a partial nucleic acid sequence for human interferon-like polypeptide and another genomic clone (*i.e.*, clone 6) was determined to contain a full-length nucleic acid sequence for human interferon-like polypeptide (*see* page 62). The amino

acid sequence of human interferon-like polypeptide was determined from the latter nucleic acid sequence.

4. The dates on the laboratory notebook pages have been redacted from the photocopies. However, the dates are before July 21, 1998, the date on which U.S. Provisional Application No. 60/093,643 was filed, from which U.S. Application No. 09/487,792 claims the benefit of priority, from which U.S. Patent No. 6,433,145 issued on August 13, 2002.

5. Also accompanying this Declaration are photocopies of ten (10) pages from a Research Summary showing that the invention disclosed and claimed in the instant patent application was diligently reduced to practice. Specifically, the photocopies of the Research Summary show that experiments were performed in order to determine the function of protein encoded by the nucleic acid sequence described in paragraph 3 above, and that once the function of the protein had been determined, a Research Summary was prepared and submitted to the legal department of Amgen Inc., the assignee of the instant application. More particularly, photocopies of the Research Summary show that several versions of the human and rat IFN-L proteins were produced in a mammalian expression system (*see* page 7) and that rat IFN-L:Fc fusion protein treatment of several cell lines was found to cause phosphorylation of cellular proteins (*see* page 10).

6. The dates on the Research Summary pages have been redacted from the photocopies.

7. We hereby declare further that all statements made herein by each of us to our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: June 10, 2004

Signed: \_\_\_\_\_  
Andrew A. Welcher

\_\_\_\_\_  
Duanzhi Wen

\_\_\_\_\_  
Michael Kelly

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

IFN

34

ge No. \_\_\_\_\_

hrpe3-00078-F6.

① related to rIFN  $\beta$  (30%).② ① In Certain lot of pancreas mRNA (human) Northern.  
(J. Cao).

③ Screening of pancreas cDNA (human) Library ②.

? Different lot of RNA source

? Different level of expression

Decision: ① re-screening human pancreas Library  
② determine the genomic screening  
condition

③ Is genomic screening feasible?

IFN probe  
for genomic blot

PAGE: 1

13:16

ID: CHERENCOV

0.5

USER: 2

COMMENT:

PRESET TIME :

0.50

DATA CALC :

CPM

H# :

NO

SAMPLE REPEATS:

1

PRINTER

STD

COUNT BLANK :

NO

IC# :

YES

REPLICATES :

1

RS232

OFF

TWO PHASE :

NO

AQC :

NO

CYCLE REPEATS :

1

SCINTILLATOR:

XTAL

LUMEX: NO

LOW SAMPLE REJ:

0

LOW LEVEL :

NO

HALF LIFE CORRECTION DATE:

none

ISOTOPE 1:

32P

%ERROR: 0.00

FACTOR: 1.000000

BKG. SUB:

0

SAM PGS  
NOTIME - IG#  
MIN32P  
CPM %ERRORLUMEX  
%ELAPSED  
TIME

1 \*\* -1

0.50 655.1

427775.8

0.43

0.00

0.83

4.3x10<sup>5</sup> cpm/λ

To Page No. \_\_\_\_\_

Page No. \_\_\_\_\_

PCR - Hot rIFN probe:

template	1 $\lambda$
1795-01	20 pm
1795-02	1 $\lambda$
10X PCR Buf.	10 $\lambda$
10 mM dNTPs	10 $\lambda$
<sup>32</sup> P-dCTP	5 $\lambda$
25 mM MgCl <sub>2</sub>	16 $\lambda$
Taq	1 $\lambda$
H <sub>2</sub> O to	100 $\lambda$

94°C 30sec, 50°C 30sec 74°C 1min for 40 cycles.

G-50 column purified.

count:  $4.3 \times 10^5$  cpm/ $\lambda$ .

Determine the hybridization and washing condition for genomic screening.

- ① very likely the homology of hIFN vs. rIFN is in the neighborhood of G<sub>0</sub>.
- ② The formamide should be between 25% - 30%

washing condition should start with gentle wash, then elevate the T wash. Determine a condition in which fragment is detectable while background is minor.

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From the genomic southern (D. Wen keeps all the photo) it is clear:

① There is an 1.8kb *HindIII* fragment that strongly hybridize w/ Probe.

- ② The formamide concentration can be adjusted to ~30% for relatively optimal signal vs. noise ratio.
- ③ Washing can be conducted @ ~55°C in 0.2 or 0.3X SSC, 0.1% SDS.

Wen further found in literature:

- ① IFN $\gamma$  family member is single gene, i.e. no intron.
- ② A lot of pseudo IFN gene as well.

But what about we identify a gene resemble *nrpe3-0078-F6*, also proved its expressing tissues?



Page No. \_\_\_\_\_

Screening hPancreas Library w/ Rat Probe

ITN - probe for human pancreas Library screening

PAGE: 1

ID: CHERENCOV 0.5

COMMENT: .

USER: 2

PRESET TIME : 0.50

DATA CALC : CPM

COUNT BLANK : NO

TWO PHASE : NO

SCINTILLATOR: XTAL

LOW LEVEL : NO

H# : NO SAMPLE REPEATS: 1

IC# : YES REPLICATES : 1

AQC : NO CYCLE REPEATS : 1

LUMEX: NO LOW SAMPLE REJ: 0

HALF LIFE CORRECTION DATE: none

PRINTER : STD

RS232 : OFF

ISOTOPE 1: 32P %ERROR: 0.00 FACTOR: 1.000000 BKG. SUB: 0

SAM NO	POS	TIME MIN	IC#	32P CPM	%ERROR	LUMEX %	ELAPSED TIME
1	**	-1	0.50	606.2	827666.2	0.31	0.01 0.91

PCR as on p. 35.

Library screening

- 1x 10<sup>6</sup> clone on 20 plates

- 30% formaldehyde. 5x SSC. 42°C O/N

- Wash: 2x SSC. 0.1% SDS 30min @ 50°C

- O/N exposure

And

There is no double positive clones.

To Page No. \_\_\_\_\_

Issued &amp; Understood by me,

Date

Invented by

Date

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

ITN

38

e No. \_\_\_\_\_

Screening human fibroblast genomic Library.

Library: human lung fibroblast genomic Library in  
Fix II vector (Stratagene).1x10<sup>6</sup> independent clones on nitro-  
cellulose membrane.

PCR probe: as described on p.35

198  
ITN probe for genomic Screening

PAGE: 1

12:47

D:CHERENCOV 0.5

IER: 2 COMMENT:

RESET TIME : 0.50

ITA CALC : CPM

IUNT BLANK : NO

ID PHASE : NO

INTILLATOR: XTAL

JW LEVEL : NO

H# : NO SAMPLE REPEATS: 1

IC# : YES REPLICATES : 1

AQC : NO CYCLE REPEATS : 1

LUMEX: NO LOW SAMPLE REJ: 0

HALF LIFE CORRECTION DATE:

PRINTER : STD

RS232 : OFF

none

ISOTOPE 1: 32P %ERROR: 0.00 FACTOR: 1.000000 BKG. SUB: 0

AM	POS	TIME	IC#	32P	LUMEX	ELAPSED
JO		MIN		CPM %ERROR	%	TIME

1	**	-1	0.50	632.0	704931.5	0.34	0.01	0.89
---	----	----	------	-------	----------	------	------	------

To Page No. \_\_\_\_\_

Page No. \_\_\_\_\_

Life Library to Nitrocellulose membrane.

① 5 min denature, 5 min neutralization. 15 Sec 2X SSC

② 80°C bake 1 hr.

③ pre-hyb: 30% Formamide  
5X SSC  
2X Denhart's  
100 µg/ml ssDNA  
0.2% SDS  
2 mM EDTA  
0.1% Pyrophosphate

42°C O/N

④ Wash.

At R.T. 1X SSC, 0.1% SDS. 2x 30 min

At 55°C 0.2X SSC, 0.1% SDS 15 min

-80°C BioMax Kodak film O/N at -80°C

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

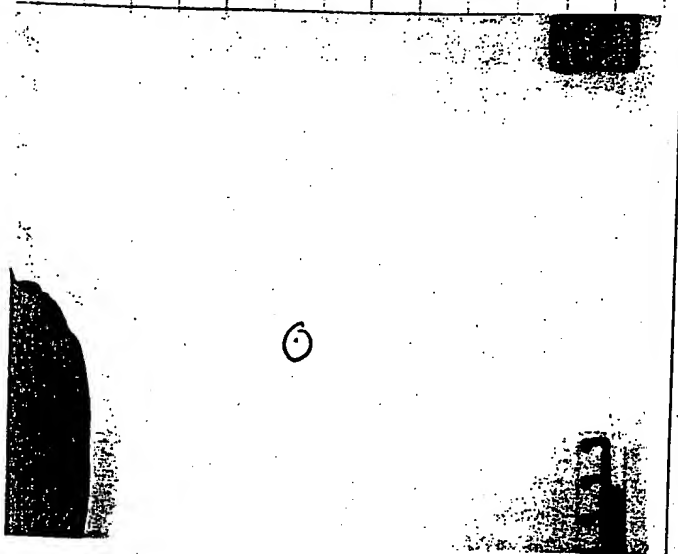
TITLE \_\_\_\_\_

IFN

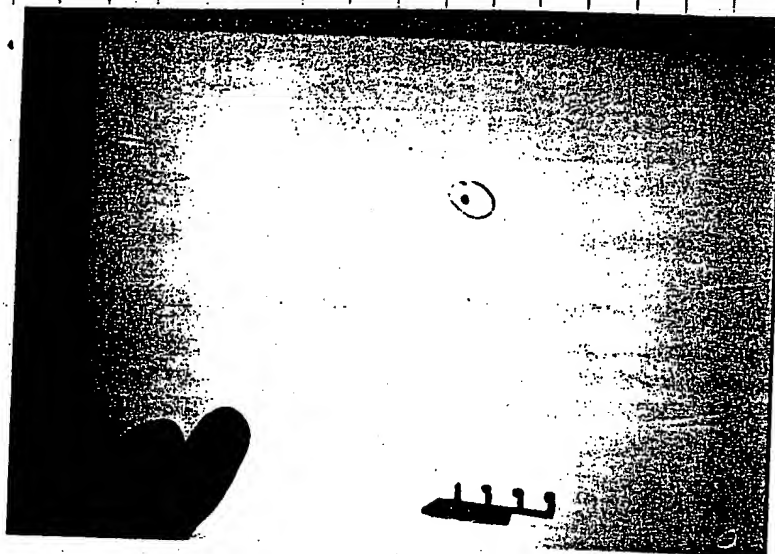
40

ie No. \_\_\_\_\_

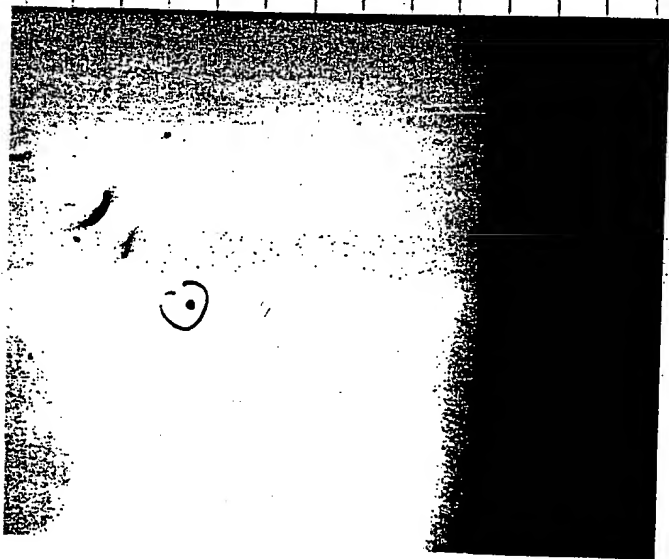
Primary screening Result.



# 2



#6



1) pick up the  $\phi$  colony with  
SM elution 37°C 2 hrs.

2)  $1/500$  dilution for secondary

3) double-lift secondary  
screening membrane.

# 7. All double positive

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Page No. \_\_\_\_\_

2nd screening 5 h IZN

4/19/98  
IZN probe

PAGE: 1

ID: CHERENCOV 0.5

USER: 2

COMMENT:

20:13

PRESET TIME : 0.50

DATA CALC : CPM

COUNT BLANK : NO

TWO PHASE : NO

SCINTILLATOR: XTAL

LOW LEVEL : NO

H# : NO SAMPLE REPEATS: 1

IC# : YES REPLICATES : 1

AQC : NO CYCLE REPEATS : 1

LUMEX: NO LOW SAMPLE REJ: 0

HALF LIFE CORRECTION DATE:

PRINTER : STD

RS232 : OFF

none

ISOTOPE 1: 32P %ERROR: 0.00 FACTOR: 1.000000 BKG. SUB: 0

SAM NO	POS	TIME MIN	IC#	32P CPM	%ERROR	LUMEX %	ELAPSED TIME
1	** -1	0.50	615.7	458314.7	0.42	0.01	0.86

Hybridization: 30% formaldehyde  
5xSSC  
42°C o/n.

Wash: 1xSSC, 0.1% SDS R.T. 30min

0.2xSSC, 0.1% SDS 55°C 15min

To Page No. \_\_\_\_\_

essed &amp; Understood by me,

Date

Invented by

Date

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

IN

42

ge No. \_\_\_\_\_

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

pick-up 2nd clone . elute with SM.

prepare for phage DNA preparation.

Page No. \_\_\_\_\_

~~XXXXXXXXXX~~  $\phi$  DNA preparation protocol,

aration of lamdan phage DNA  
 row up the phage (on plates or in liquid medim).  
 llecte the supernatant and spin down with 3000rpm.  
 dd DNaseI (10u/ml) and RNase (20 $\mu$ g/ml) incubate 30 min at  
 c degree.  
 d 1/3 volume 30%PEG 6000 in 3M NaCl put in ice for 1 hr or at 4  
 degree overnight.  
 12000g centrifugation 20 min  
 discard supernatant and interverte the tube on papers to remove  
 3 completely.  
 d 1/10-1/5 of origin volume T10E1 to suspend the phage  
 ticles.  
 d 0.5M EDTA to a final concentration of 20mM and proteinaseK  
 mg/ml, incubate 1hr in 55c degree waterbath.  
 id 5%CATB in 0.5M NaCl to a final concentration of 0.1% and put  
 65c degree for 5 min.  
 sin down and dissolve in 1.2M NaCl.  
 Add 2.5vol. of ethanol and spin down.  
 discard the ethanol and wash once with 70% ethanol.  
 r dry the pellets and dissolve in T10E1 in ice 30 min. then at  
 ic degree for 10min. don't put on RT. stock in 4c degree.

- phage has to be amplified once before proceeding to  $\phi$  DNA prep. on a 100mm plate. 10ml  $\phi$  + top agar  $\rightarrow$  o/n 37°C.

SM elute

- on LB/Agarose plate, grow  $\phi$  o/n @ 37°C

- elute w/ SM 2 1/2 hrs @ 3-7.

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Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

IFN

44

e No. \_\_\_\_\_

BECKMAN DU-600

Human Genomic IFN like clone  
Φ prep.Date: \_\_\_\_\_  
Time: 23:19leic Acid  
adSamples

Method

SaveClear

Print

Quit

Results file: A:\WORK\_RES

Method name: A:\DEFAULT

Assay type: General Ratio and Concentration

Formula setup: VIEW

Sampling device: None

Read average time: 0.50 sec

Units: UG/UL

Background Correction: [No ]

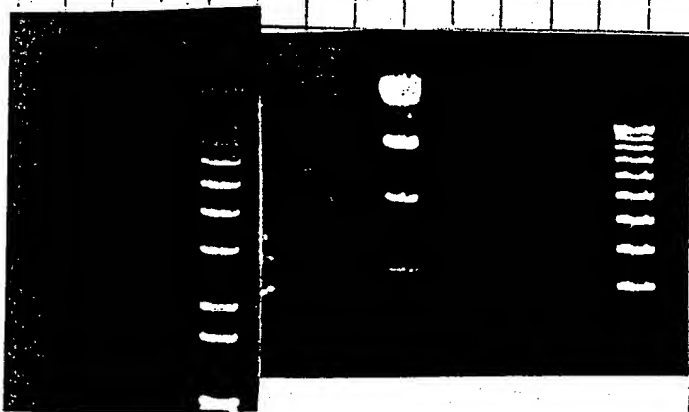
Concentration: [Yes]

Peak Pick: [No ]

↓ ↑

Sample	abs 260.0 nm	abs 280.0 nm
#2	0.1428	0.0774
#6	0.3206	0.1775
#7	0.1731	0.0913

260.0 nm	280.0 nm	x100 SSRNA UG/UL	X100 dsDNA UG/UL
280.0 nm	260.0 nm		
1.8453	0.5419	0.5711	0.7139
1.8066	0.5535	1.2823	1.6029
1.8962	0.5274	0.6922	0.8653



#2: 143 ng/λ  
 #6: 32.1 ng/λ  
 #7: 17.3 ng/λ

Set-up NotI digestion to  
 release insert in pIX.

20 ng Φ DNA

1.0x H

NotI (HC) 40u x 2.5 = 100

H<sub>2</sub>O

ON @ 37°C incubator.

To Page No. \_\_\_\_\_



Page No. \_\_\_\_\_

IFN

~~where~~ where is the IFN fragment?

The two ← indicated on p. 44 insert of interest?

Repeat Not I digestion; run a small agarose 0.5% gel.

Transfer and Southern to determine the band

Hyb. 30% Formamide, wash: 0.2xSSC, 0.1% SDS.  
55°C 15min

Results on p. 46

BECKMAN DU-600

Date: \_\_\_\_\_  
Time: 16:18

Nucleic Acid  
ReadSamples

Method

SaveClear

Print

Quit

Results file: A:\WORK\_RES

Method name: A:\DEFAULT

Assay type: General Ratio and Concentration

Units: UG/UL

Formula setup: VIEW

Background Correction: [No]

Sampling device: None

Concentration: [Yes]

Read average time: 0.50 sec

Peak Pick: [No]

Sample ID	1/20		260.0 nm	280.0 nm	x100 ssRNA	X100 dsDNA
	abs 260.0 nm	abs 280.0 nm	280.0 nm	260.0 nm	UG/UL	UG/UL
1 #2	0.0059	0.0027	2.1876	0.4571	0.0235	0.0293
2 #6	0.0082	0.0043	1.8895	0.5292	0.0326	0.0408
3 #7	0.0085	0.0046	1.8522	0.5399	0.0338	0.0423
4						

#2 5.9 x 50 x 20 = 6 ng/λ  
#6 8.2 x 1000 = 8 ng/λ  
#7 8.5 x 1000 = 8.5 ng/λ

Ligation: 10 ng cut vect.  
+ 6 λ Insert  
+ 2 λ 10x Buff  
5U T4 ligase

Assessed &amp; Understood by me,

Date

Invented by

Date

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

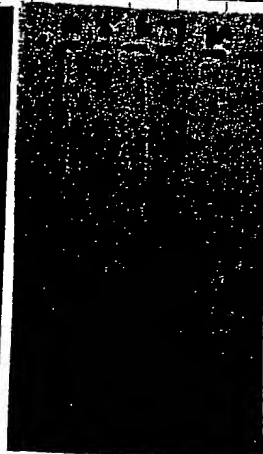
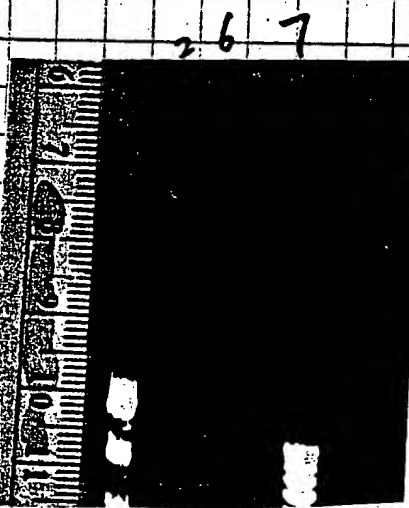
ITN

46

Page No. \_\_\_\_\_

[REDACTED]

267



[REDACTED] Gel purifying ~~ITN~~ genomic fragment

267

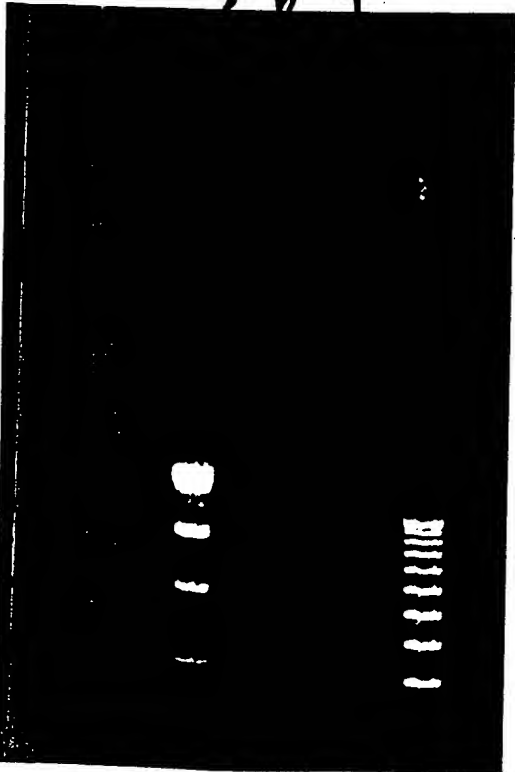


IMAGE SIZE (640 x 480 x 8).  
INT PERIOD = 0.36 SEC.  
ACQUIRED [REDACTED]  
STROBOSCOPE EYE II [REDACTED] 13:52:19

267

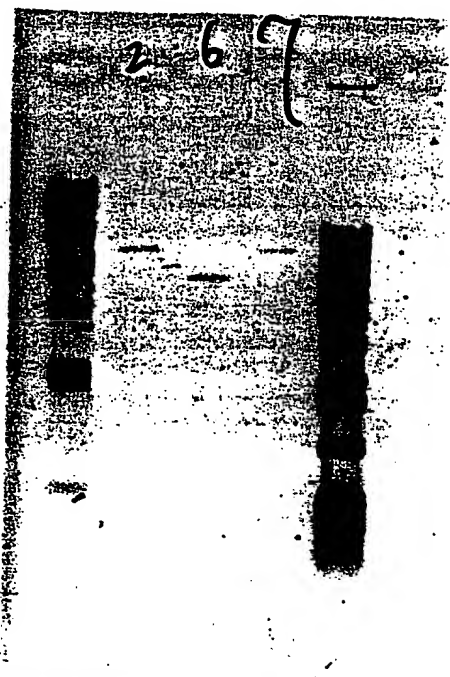


IMAGE SIZE (640 x 480 x 8).  
INT PERIOD = 0.37 SEC.  
ACQUIRED [REDACTED]  
STROBOSCOPE EYE II [REDACTED] 16:04:28

I have read and understood by me.

Date \_\_\_\_\_

Invented by \_\_\_\_\_

Date \_\_\_\_\_

*W. Chen*

Page No. \_\_\_\_\_

pSV. Sport/NotI genomic fragment

BECKMAN DU-600

Nucleic Acid  
Read Samples

Method

Results file: A:\WORK\_RES

Assay type: General Ratio and Concentration

Formula setup: VIEW

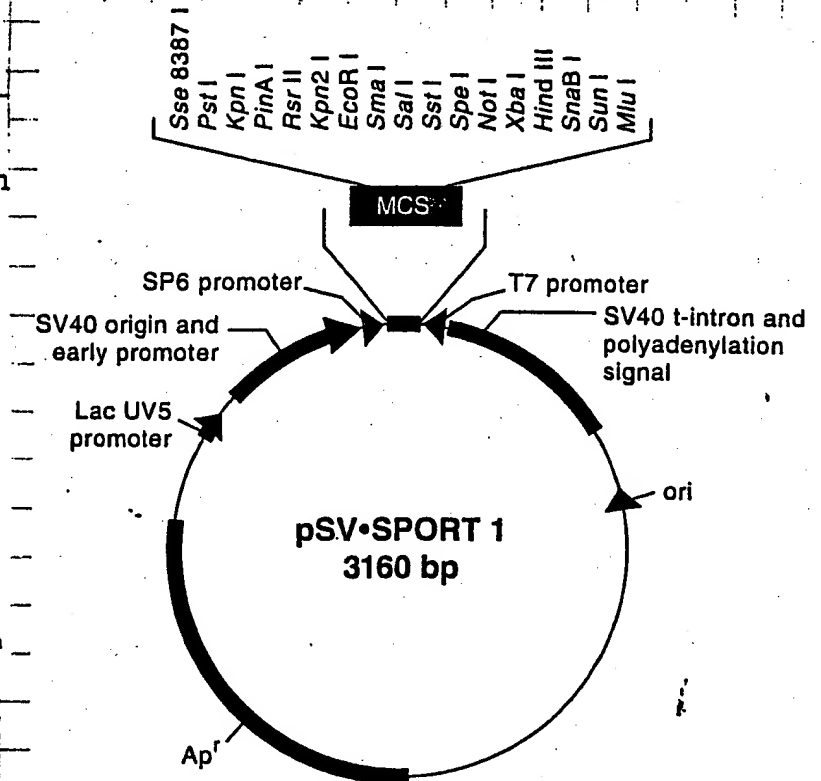
Sampling device: None

Read average time: 0.50 sec

Sample ID	abs 260.0 nm	abs 280.0 nm
1 pSV.pSport	0.0107	0.0039
2 pSV.pSport	0.0204	0.0093
3 pSV.pSport		

1 pSV.pSport  
2 pSV.pSport  
3 pSV.pSport

pSV.pSport/NotI 20 ng/μl



0.8% TAE - agarose gel / NotI digestion

STRATAGENE EAGLE EYE II 12:46:39

SIZE (640 x 480 x 8).

PERIOD = 8.13 SEC.

RED

RED

RED

RED

RED

RED

RED

RED

RED

RED

RED

RED

RED

RED

RED

RED

RED

RED

RED

RED

RED

RED

RED

RED

RED

RED

RED

RED

RED

RED

RED

RED

RED

RED

RED

RED

RED

RED

RED

RED

miniprep DNA 5μl

10X H 1μl

NotI 0.5μl (10u)

H<sub>2</sub>O 3.5μl

Total 10μl @ 37°C 1 1/2 hrs.

← Insert  
← pSV.pSport

To Page No. \_\_\_\_\_

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

1.1. 17N

48

ge No. \_\_\_\_\_

Set-up genomic clone Analysis.

5 $\lambda$  mini-prep DNA  
 1 $\lambda$  10x Buffer (BMB)  
 0.5 $\lambda$  EZ  
 3.5 $\lambda$  H<sub>2</sub>O

10 $\lambda$  @ 37°C 1 1/2 hrs.

Restriction EZs:

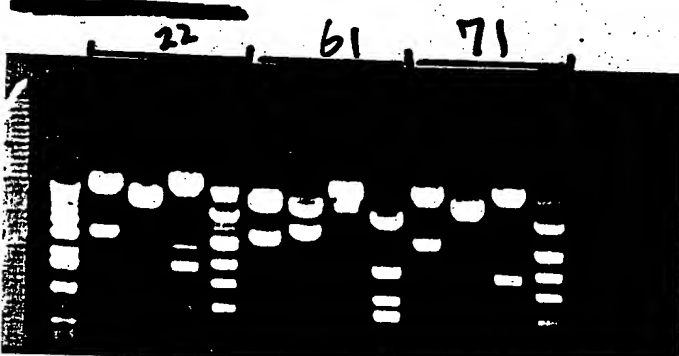
present on MCS of psu.pspart

NotI, EcoRI, XbaI &amp; HindIII

87% TAE gel.

STRATAGENE EAGLE EYE II 19:18:42

540 x 480 x 8).  
 ID = 0.46 SEC.



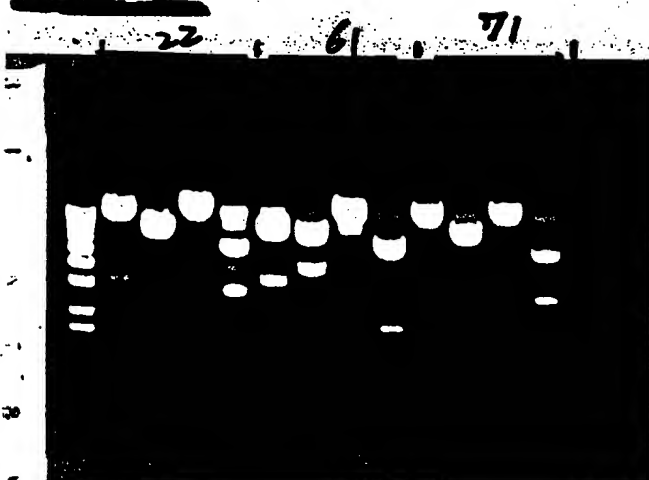
22 and 71 are identical independent clones. but not 61.

	22	61	71
EcoRI	0	2	0
XbaI	3	2	3
HindIII	7/4	7/4	7/4

Is that why initially EcoRI digestion on genomic DNA didn't light up by rmp3 probe?

STRATAGENE EAGLE EYE II 19:35:05

540 x 480 x 8).  
 ID = 0.66 SEC.



22  
61  
71  
HindIII  
XbaI  
EcoRI  
NotI

NEX H NEX H

Transfer this gel w/ alkaline transfer.



\* HindIII incomplete digestion

To Page No. \_\_\_\_\_

Page No.           **Washing Southern Blot.**

Hybridization: 30% Formamide, 5x SSC, 2x Denhart's  
 10% SSDNA, 0.2% SDS, 2mM EDTA, 0.1% pyrophos  
 (No  $\text{NH}_2\text{PO}_4$ )  
 42°C, 3 hrs. + 5% Dextran Sulfate for Hyb.

PCR - Hot probe: Template 1  $\lambda$  (20ng)  
 1795-01 1  $\lambda$  (20pm)  
 1795-02 1  $\lambda$  (20pm)  
 10mM dNTP 10  $\lambda$  (dCTP @ 0.1mM)  
 $^{32}\text{P}$ -dCTP 5  $\lambda$   
 10x PCR buf. 10  $\lambda$   
 25mM  $\text{MgCl}_2$  16  $\lambda$  (4mM final)  
 Taq 1  $\lambda$   
 H<sub>2</sub>O to 100  $\mu$ l control: + 60  $\mu$ l H<sub>2</sub>O  
 with 10mM dNTP.

5/7/98 RTN probe

PAGE: 1

22:04

ID: CHERENCOV 0.5

USER: 2

COMMENT:

PRESET TIME :	0.50	H# :	NO	SAMPLE REPEATS:	1	PRINTER :	STD
DATA CALC :	CPM	IC# :	YES	REPLICATES :	1	RS232 :	OFF
COUNT BLANK :	NO	AQC :	NO	CYCLE REPEATS :	1		
TWO PHASE :	NO	LUMEX:	NO	LOW SAMPLE REJ:	0		
SCINTILLATOR:	XTAL	HALF LIFE CORRECTION DATE:	none				
LOW LEVEL :	NO						

ISOTOPE 1: 32P %ERROR: 0.00 FACTOR: 1.000000 BKG. SUB: 0

SAM NO	POS	TIME MIN	IC#	32P CPM	%ERROR	LUMEX %	ELAPSED TIME
1	** -1	0.50	592.9	528289.8	0.39	0.01	0.86

1  $\mu$ l count.

Washing: • 1 x SSC, 0.1% SDS @ RT 1 hr  
 0.2 x SSC, 0.1% SDS @ 55°C 15 min  
 exp. @ -80°C 0/N.

To Page No.           Issued & Understood by me,                     Date           Invented by           Date

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TITLE

17N

50

le No. \_\_\_\_\_

h mrep-3 cloning: Subclone genomic fragment.  
Set-up digestion.

70λ mini-prep DNA  
8λ 10x B Buffer (BMB)  
2λ BamHI (20u)  
80λ total @ 37°C 5 hrs

8λ run gel (with dye)  
◀ indicates positive band in  
Southern Blot with mrep-3  
probe (p. 48).

92λ (with dye) run a 8% TAE gel.

Qiagen Gel Purification Kit



0.8% TAE-agarose gel

(Hind III fragments)

BECKMAN DU-600

Date: \_\_\_\_\_

Time: 21:25

leic Acid  
adSamples

Method

SaveClear

Print

Quit

Results file: A:\WORK\_RES

Method name: A:\DEFAULT

ssay type: General Ratio and Concentration

Units: UG/UL

Formula setup: VIEW

Background Correction: [No]

Sampling device: None

Concentration: [Yes]

Avg average time: 0.50 sec

Peak Pick: [No]

1/10 dilu

sample	abs	abs	260.0 nm	280.0 nm	x100	X100
					ssRNA	dsDNA
			260.0 nm	280.0 nm	UG/UL	UG/UL
#61	0.0168	0.0093	1.7933	0.5576	0.0670	0.0838
#71	0.0201	0.0112	1.7963	0.5567	0.0803	0.1003

#61 0.0168 x 50 x 20 ≈ 1718/λ  
#71 0.0201 x 50 x 20 ≈ 2010/λ

No. 61, 71

61, 71 insert  
is probably  
same (identical  
size, Hind III  
fragment size)

To Page No. \_\_\_\_\_

&amp; Understood by me.

Date

Invented by

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Ligation:

3  $\lambda$  dephosphorylated psv-pspmt (17ng)  
 1  $\lambda$  10x Lig. Buffer (BMB)  
 1  $\lambda$  T4 DNA Ligase (BMB)  
 5  $\lambda$  Insert (85 ~ 100ng)  
 10  $\lambda$

Control: + 5  $\mu$ l H<sub>2</sub>O in vector control.

14°C 1 hr

Transformation: 140  $\lambda$  DH10 $\alpha$  ElectroMax cell  
 + 1.5  $\lambda$  Ligation Mix

6 transformation clones/each  
for miniprep.

STRATAGENE EAGLE EYE II 13:20:23

E SIZE (640 x 480 x 8).  
 PERIOD = 0.36 SEC.  
 IRED

HindIII

61

72

Digest w/ HindIII

5  $\lambda$  DNA1  $\lambda$  10x B (BMB)0.5  $\lambda$  HindIII (5u)3.5  $\lambda$  H<sub>2</sub>O10  $\lambda$  total 37°C 1/2 hr

2% TAE gel

All contain insert. (1.8 kb)

Further Analysis w/

KpnI, PstI, SmaI (BMB)

5  $\lambda$  DNA1  $\lambda$  10x Buffer0.5  $\lambda$  EZ (5u)3.5  $\lambda$  H<sub>2</sub>O10  $\lambda$  37°C 1/2 hr

So no additional EZ cut in the insert.

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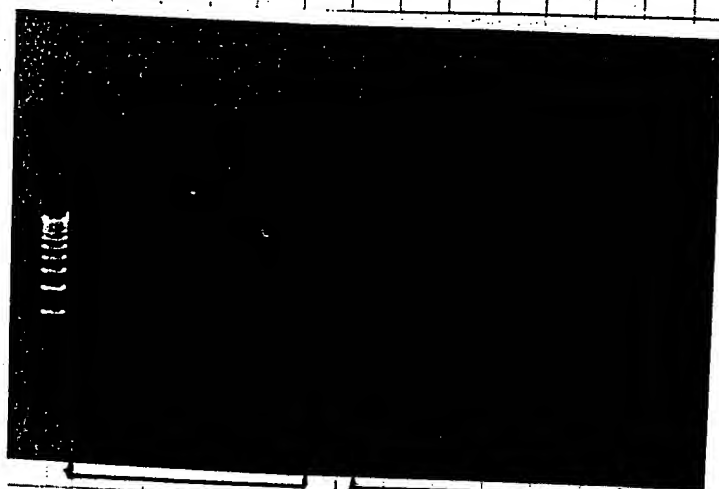
Book No. \_\_\_\_\_

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ZFN

52

age No. \_\_\_\_\_



Transfer to N.C. for future reference

61

71

Sequencing Request

D	Clone	Requestor		Status	Submit	Rcvd
9808902	hgmrep3-6.1	Wen	Chen	Pending	██████	00/00/00
9808903	hgmrep3-6.2	Wen	Chen	Pending	██████	00/00/00
9808904	hgmrep3-6.5	Wen	Chen	Pending	██████	00/00/00
9808905	hgmrep3-7.1	Wen	Chen	Pending	██████	00/00/00
9808906	hgmrep3-7.2	Wen	Chen	Pending	██████	00/00/00
9808907	hgmrep3-7.2 X deleted!	Wen	Chen	Pending	██████	00/00/00
9808908	hgmrep3-7.3	Wen	Chen	Pending	██████	00/00/00

3-6.1 } identical insert in same orientation  
 3-6.5 }  
 3-7.1 }

3-6.2 } identical insert in reverse orientation  
 3-7.2 }



Page No. \_\_\_\_\_

mCamp2 Southern: Human Genomic DNA

probe: mCamp2 template 2λ (long)  
 1582-15 (5' primer) 2λ (20pm)  
 1536-79 (3' primer) 2λ (20pm)  
 10x Buffer 10λ  
 10mM dNTP (dCTP @ 0.1mM) 10λ  
 α-<sup>32</sup>P dCTP 5λ  
 25mM MgCl<sub>2</sub> 16λ  
 Taq (BMB) 1λ  
 H<sub>2</sub>O 52λ

Cold control + 10λ 10mM dNTP + 52 extra H<sub>2</sub>O  
 94°C 30sec → 60°C 30sec → 45°C 45 cycles  
 1min

PAGE: 1

ID: CHERENCOV 0.5

USER: 2

COMMENT:

16:06

PRESET TIME : 0.50

DATA CALC : CPM

COUNT BLANK : NO

TWO PHASE : NO

SCINTILLATOR: XTAL

LOW LEVEL : NO

H# : NO SAMPLE REPEATS: 1

IC# : YES REPLICATES : 1

AQC : NO CYCLE REPEATS : 1

LUMEX: NO LOW SAMPLE REJ: 0

HALF LIFE CORRECTION DATE:

PRINTER : STD

RS232 : OFF

none

ISOTOPE 1: 32P %ERROR: 0.00 FACTOR: 1.000000 BKG. SUB: 0

SAM NO	POS	TIME MIN	IC#	32P CPM	%ERROR	LUMEX %	ELAPSED TIME
1	** -1	0.50	602.8	1115564	0.27	0.02	0.95

1λ 1.1 x 10<sup>6</sup> cpm/λ

Cold control.

6/ primary Screening:

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Chy 22

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e No. \_\_\_\_\_

Hybridization in 30% Formamide

30% Formamide  
5X SSC

2X Denhardt's

10 µg/ml ssDNA

0.2% SDS

2 mM EDTA

0.1% Pyrophosphate

H<sub>2</sub>O

to 100 ml

Stock  
30 ml  
25 ml4 ml  
1 ml

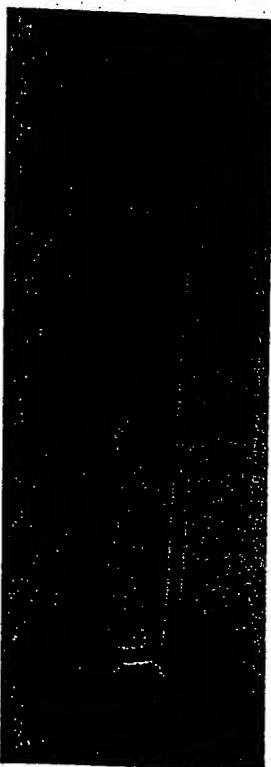
1 ml

0.4 ml

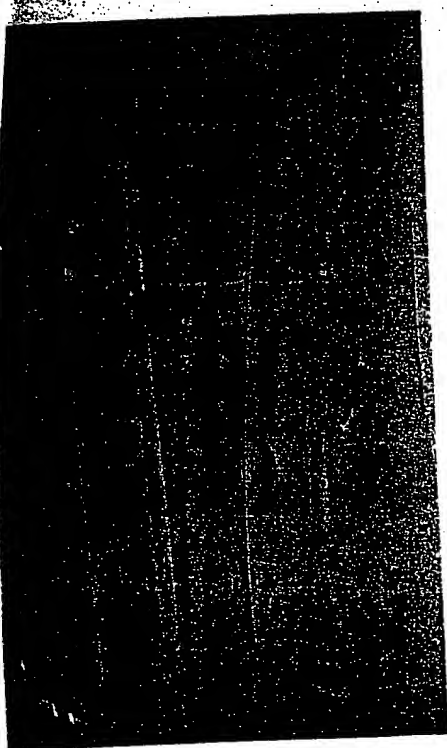
1 ml

37.6 ml

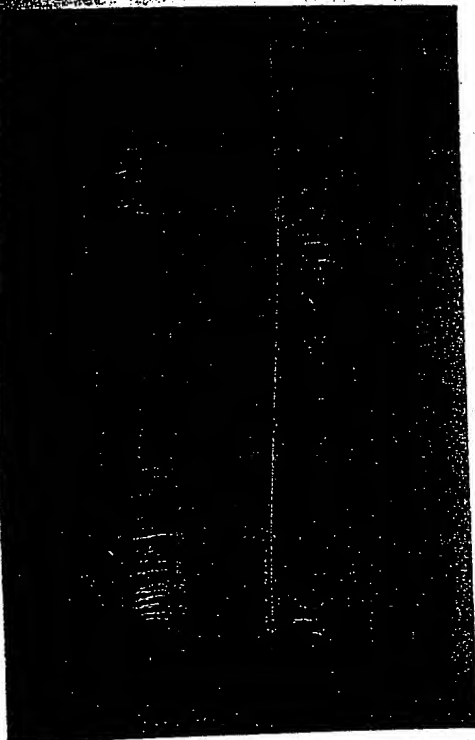
42°C 0.1N . stripe rehyb. 1



20X Formamide, 0.5X Wash SSC, 24 hrs  
IMAGE SIZE (640 x 480 x 8).  
REAL-TIME ACQUIRE.



20X Formamide, 0.5X Wash SSC, 24 hrs  
IMAGE SIZE (640 x 480 x 8).  
REAL-TIME ACQUIRE.



20X Formamide, 0.5X Wash SSC, 24 hrs  
IMAGE SIZE (640 x 480 x 8).  
REAL-TIME ACQUIRE.

STRATAGENE EAGLE EYE II 15:07:30

action 30%  
PC (Formamide)  
SSC 0.2X  
SDS 0.1%  
c exp 24 hrs

20%  
0.5X  
0.1%  
24 hrs

20%  
0.5X  
0.1%  
24 hrs

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E

IRN

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5

REVERSE-COMPLEMENT of: 9808902.Con check: 3852 from: 1 to: 1163  
When  
DNA-hgmrep3-6.1 p=890-24 end  
assembled by JK: [REDACTED]  
9808902.con

With 1 enzymes: HINDIII

[REDACTED] 17:01 ..

1 CTGAGAAGAGTCACCTGGCAAAATCTGAGACATCTGAGTAGTATGAGCAATTCATTTCCT  
-----+-----+-----+-----+-----+-----+ 60  
GACTCTTCTCAGTGGACCGTTTTAGACTCTGTAGACTCATCATACTCGTTAAGTAAAGGA

a L R R V T W Q N L R H L S S M S N S F P -

61 GTAGAATGTCTACGAGAAAACATAGCTTTTGAGTTGCCCAAGAGTTTCTGCAATACACC  
-----+-----+-----+-----+-----+ 120  
CATCTTACAGATGCTCTTTTGTATCGAAAACCAACGGGGTTCTCAAAGACGTTATGTGG

a V E C L R E N I A F E L P Q E F L Q Y T -

121 CAACCTATGAAGAGGGACATCAAGAAGGCCTTCTATGAAATGTCCCTACAGGCCTTCAAC  
-----+-----+-----+-----+-----+ 180  
GTTGGATACTTCTCCCTGTAGTTCTTCCGGAAGATACTTTACAGGGATGTCCGGAAGTTG

a Q P M K R D I K K A F Y E M S L Q A F N -

181 ATCTTCAGCCAACACACCTTCAAATATTTGAAAGAGAGACACCTCAAACAAATCCAAATA  
-----+-----+-----+-----+-----+ 240  
TAGAAGTCGGTTGTGTGGAAGTTTATAACCTTCTCTCTGTGGAGTTTGTAGGTTTAT

a I F S Q H T F K Y W K E R H L K Q I Q I -

241 GGACTTGATCAGCAAGCAGAGTACCTGAACCAATGCTTGGAGGAAGACGAGAATGAAAAT  
-----+-----+-----+-----+-----+ 300  
CCTGAAGTATGCTTCTCTCATGGACTTGGTTACGAACCTCTCTCTCTTACTTTTA

a G L D Q Q A E Y L N Q C L E E D E N E N -

301 GAAGACATGAAAGAAATGAAAGAGAATGAGATGAAACCTCAGAAGCCAGGGTCCCCAG  
-----+-----+-----+-----+-----+ 360  
CTTCTGTACTTTCTTTACTTTCTTACTTCTACTTTGGGAGTCTCGGTCCAGGGGGTCT

a E D M K E M K E N E M K P S E A R V P Q -

361 CTGAGCAGCCTGGAAGTGGAGATATTTCCACAGGATAGACAATTTCTGAAAGAAAAG  
-----+-----+-----+-----+-----+ 420  
GACTCGTCCGACCTTGACTCCTCTATAAAGGIGTCTATCTGTTAAAGGACTTCTTTTC

a L S S L E L R R Y F H R I D N F L K E K -

AAATACAGTACTGTGCTGGGAGATTGTCCGAGTGGAAATCAGAAGATGTTGTATTAC

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IFN

## TITI E

56

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961 -----+-----+-----+-----+-----+----- 102

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177N

a T C S L E S I L L H F P P P A R G E K G -

1021 TGACATTTCIGGCCCATTTCCCTCTCAGCTTGGTTTGTGTTGAATTGATGCTTGTGGAATG  
-----+-----+-----+-----+ 1080  
ACTGTAAAGACCGGGTAAAGGAAGAGTGAACCAAACAACTTAACTACGAACACCTTAC

INTRON>

INTRON>

a \* H F W P I S F S A W F V \* I D A C G M -

1081 GIATTTTCATTACTTTAAGAGTGAAGATCCATAGTGAATTTGGATGGATGGTTGAATTAGA  
-----+-----+-----+-----+ 1140  
CATAAAGTAATGAAATTTCTCACTTCTAGGTATCACTTTAACCTACCTACCAACTTAATCT

a V F H Y F K S E D P \* \* N W M D G \* I R -

HindIII

1141 CGACCATTAAAGCTTACGTACGCG  
-----+-----+----- 1163  
GCTGGTAATTGCAATGCATGCGC

a R P L S L R T -

Enzymes that do cut:

HindIII

Untitled-5	MTLKYLWLVA LVALYISPIQ SQNCVYLDHT ILE	17
Untitled-3	LRH MSNS VE	50
Consensus	.....	50
Untitled-5	RNLIA	66
Untitled-3	KLITTE	100
Consensus	.....	100
Untitled-5	H KQOITD QAEYLNOL E	116
Untitled-3	RER RS F KVVQQAEM V	146
Consensus	.....	150
Untitled-5	RRR	164
Untitled-3	LNK	191
Consensus	.....	198

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Fomega  
clone

nsensus

MALLFPLLA LVMTSYSPVG SLGCDLPQNH GLLSRNTVL HQMRRISF  
-----LRR-- --VTWQNRH SSMSNSFV

50

21

50

Fomega  
clone

nsensus

IKKRRRDER FDMVKGS LQAHMSVL HEMLOQIFSL HTERSSAA  
EURENIARE LQDFLOYT PMRDKKAF YEMSLQAFNI --SQHTFKY

100

70

100

Fomega  
clone

nsensus

NMTLDQLHT HQHLOHLE TQILQVVGEG -----SAGAISS  
KERHKKIQI LQDAEYEN QLEEDENEN EDMKEMKENE MKPSEARVPQ

138

120

150

Fomega  
clone

nsensus

-PILIRRYM QGRVY LQAHMSVL HEMLOQIFSL STNMQERLRS  
LSSILIRRYM HRFONF LQAHMSVL HEMLOQIFSL STNMQERLRS  
FYKFTALFR

187

170

200

Fomega  
clone

nsensus

KDRDLGSS  
-----  
K-----

195

171

208

IFN

Fomega  
clone

nsensus

MTLKYLWLV LVALYISPIQ SQNCVYLDH-- --TILENKL SSIRTTFL  
MALLFPLLA LVMTSYSPVG SLGCDLPQNH GLLSRNTVL HQMRRISF  
-----LRR-- --VTWQNRH SSMSNSFV  
M.L....L.A LV....SP.. S..C.L.... --....N..L SSMR...F.

47

50

21

50

IFN

Fomega  
clone

nsensus

FKKITTDE FDEILLYV HMKDKAVT YHISSLALII SLKDTISLA  
IKKRRRDER FDMVKGS LQAHMSVL HEMLOQIFSL HTERSSAAW  
EURENIARE LQDFLOYT PMRDKKAF YEMSLQAFNI --SQHTFKYW  
K...DE F...L.Y... D.K.V. YEMS.QAF.I .....W

97

100

70

100

IFN

Fomega  
clone

nsensus

TEERERIRS FKNVQAR FVVEENKN TEEDSTSQH-- --PHSEGFK  
NMTLDQLHT HQHLOHLE TQILVVGEG E-----SAGAISS  
KERHKKIQI LQDAEYEN QLEEDENEN EDMKEMKENE MKPSEARVPQ  
.E...QI... L...C...L... ENEN E..... --PS.....

144

138

120

150

IFN

Fomega  
clone

nsensus

-VYIRRYM QGRVY LQAHMSVL HEMLOQIFSL STNMQERLRS  
-PILIRRYM QGRVY LQAHMSVL HEMLOQIFSL STNMQERLRS  
LSSILIRRYM HRFONF LQAHMSVL HEMLOQIFSL STNMQERLRS  
FYKFTALFR

191

187

170

200

IFN

Fomega  
clone

nsensus

KDRDLGSS  
K-----  
K-----

191

195

171

208

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E

Ratio: 2.484 Gaps: 4  
Percent Similarity: 63.383 Percent Identity: 63.383

Match display thresholds for the alignment(s):

| = IDENTITY

: = 5

. = 1

9808902.Con x Mrpe3-00078-F6-Wz.Ctg [REDACTED] 13:52 ..

1152 CACCTGGCAAATCTGAGACATCTGAGTAGTATGAGCAATTCATTTCCTG 1103

|| || | |||| | || | || || | || | || | || || |

159 CATCTTGGAAAACATGAACTTCTGAGCAGCATCAGGACCACCTTTCCCT 208

1102 TAGAATGTCTACGAGAAAACATAGCTTTTGAGTTGCCCAAGAGTTTCTG 1053

|| || || || || | || | || || || || || || || || ||

209 TAAGATGTCTAAAGATATCACGGATTTTGAGTTTCTCAAGAGATTCTG 258

1052 CAATACACCCAACTTATGAAGAGGGACATCAAGAAGGCCTTCTATGAAT 1003

| || | || | || | || || || || || || || || || || ||

259 CTGTACGTCCAGCATGTGAAAAAGGACATAAAGGCAGTCACCTATCATAT 308

1002 GTCCCTACAGGCCTTCAACATCTTCAGCC...AACACACCTTCAAATATT 956

|| | || | || | || || || || || || || || || || ||

309 ATCTTCTCTGGCGCTAATTATTTTCAGTCTTAAAGACTCCATCTCCCTGG 358

955 GGAAAGAGAGACACCTCAAACAAATCCAAATAGGACTTGATCAGCAAGCA 906

|| || || || || | || | || | || || || || || || ||

359 CGACAGAGGAACGCTTGAACGTATCAGATCGGGACTTTTCAAACAAGTG 408

905 GAGTACCTGAACCAATGCTTGGAGGAAGACGAGAATGAAAATGAAGACAT 856

|| | || | || || || || || || || || || || || || ||

409 CAGCAAGCTCGAGAGTGCATGGTAGACGAGGAGAACAAGA.....ACAC 452

855 GAAAGAAATGAAAGAGAATGAGATGAAACCTCAGAAGCCAG.GGTCCCC 807

| | | || | || | || || || || || || || || || || ||

453 GGAGG.....AGGACAGTACATCAACATCCTCACTCAGAGGGCTTC 495

806 CAGCTGAGCAGCCTGGAAGTGAAGAGATATTTCCACAGGATAGACAATTT 757

|| | || || || || || || || || || || || || || || ||

496 AAGGCAGTCTACCTGGAATTGAACAAGTATTTCTTCAGAATCAGAAAGTT 545

756 CCTGAAAGAAAAGAAATACAGTACTGTCCTGGGAGATTGTCCGAGTGG 707

|||| | || || || || || || || || || || || || || ||

546 CCTGGTAAATAAGAAATACAGTTTCTGTCCTGGAAGATTGTCTGGTGG 595

706 AAATCAGAAGATGTTTGTATTACTTTTACAAATT 673

|||| || || || || || || || || || || || || || || ||

596 AAATAAGAAGATGTTTCAGTATATTTTACAAACT 629

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nessed & Understood by me,

Date

Invented by

Date





IFN

Rabbit α IFN

Programme	Interferon Like Protein		
Investigator:	Duanzhi Wen		
Animal/Quant	3 rabbits		
Immunogen:	Interferon like Protein		
Protocol:	MSU Standard		
Prog start date:			
Prog end date:	Ongoing		
Comments:	Give all sera to Duanzhi.		
ANIMAL# PI		50% Titer	
#3429 5mls	30mls		30mls
3432 5mls	30mls		30mls
#3433 5mls	30mls		30mls

Programme	Interferon Like Protein		
Investigator:	Duanzhi Wen		
Animal/Quant	3 rabbits		
Immunogen:	Interferon like Protein		
Protocol:	MSU Standard		
Prog start date:			
Prog end date:	Ongoing		
Comments:	Give all sera to Duanzhi.		
ANIMAL# PI		50% Titer	
3429 5mls	30mls	1 1500	30mls 1 2000
3432 5mls	30mls	1 2500	30mls 1 3000
3433 5mls	30mls	1 5000	30mls 1 10,000
	50% Titer		
25mls	> 1 10,000		
25mls	> 1 10,000		
25mls	> 1 10,000		

62

REVERSE-COMPLEMENT of: 9808903.Con check: 57 from: 1 to: 1894

WChen

DNA=hgmrep3-6.2

assembled by JK: [REDACTED]

9808903.con

1.8 kb Human  
Zn-like  
clone seq.

1.8 kb

hu

f

With 1 enzymes: HINDIII

[REDACTED] 15:37 ..

HindIII

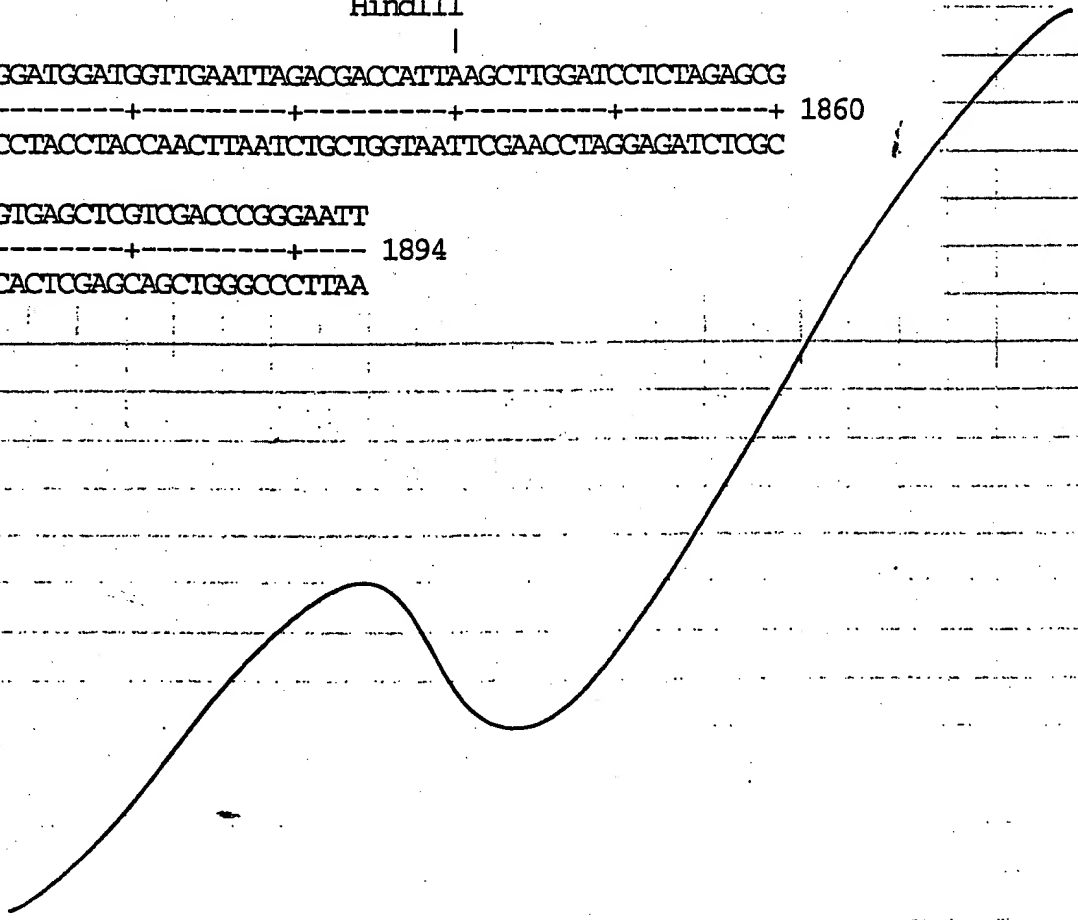
1 CGCGTACGTAAGCTTAATTTAACAAAATTGGAAAAACCTAACTATACTGTGCTCTGGTG  
 61 GCGCATGCATTICGAATTAAATTGTTTTAACCTTTTTGGATTGATATGACACGAGACCAC  
 121 ACCTAGCAATCAAATAATCACAGTCATTGGTCAATGCTATGATTAACTCAATGAGACA  
 181 TGGATCGTTAGTTTATTAGIGTCAGTAAACCAGTTACAGATACTAATTGAGTTACTCTGT  
 241 GGATGTTTGGCTATAGCACCAGGTACAAAAATATATTTTCATGAAGGATCACTCCCTCT  
 301 CCTACAAACCGATATCGTGGTCCATGTTTTTATATAAAAGTACTTCCTAGTGAGGGAGA  
 361 TATGTAATAGATTGGGTGAGTGAGTGAGTGAGTGAGTGATGGACTCACAGCTTTTGGC  
 421 ATACATTATCTAAACCCACTCACTCACTCACTCACTCACTCACTCACTGAGTGTGAAAACCG  
 481 TTCTGAAATACCTGCAATCAGTCTTGTTATGATGATTCCTTAGTGCTGGGATGGATCAT  
 541 AAAGACTTTATGGGACGTAGTCAGAACAATACTACTAAGGAATCACGACCTACCTAGTA  
 601 CCAGGCATTAAAGGTAACACGATGGTAATTCCTTTGCTCATTTTTTCAGGGAAAAAAAAG  
 660 GGTCGGTAAATTCCATTGIGCTACCATTAAGAAACGAGTAAAAAGTCCCTTTTTTTTTTTC  
 720 TTATCACTTCCAAAGTCGGCATAGTCACCGAAGTAAAAAAAAAAAAAAAAAAAAAGC  
 780 AATAGTGAAGGTTTCAGCCGATCAGTGGGCTTCATTTTTTTTTTTTTTTTTTTTTTCG  
 840 CTCAGAGGCAAAGGAAAGGGGCCAACCTTGGTTAACTGTGAAATGACGAATGAGAAAA  
 900 GAGTCTCCGTTTCCTTTCCCGGGCGTTGGAACCAATTGACACTTTACTGCTTACTCTTTT  
 960 CTCTCTGCTGAAGATATTCAAGGTATATAAAGGCACATGAAGGAAACTCAAAACATCA  
 1020 GAGGAGGACGACTTCTATAAGTCCATATATTTCCGTGTAATTCCTTTTGAGTTTGTAGT  
 1080 TTGTCATATACACATCTTCTGGATTTTTTAGCTTGCAAAAAAATGAGCACCAACCTGA  
 1140 AACAGTATATGIGTAGAAGACCTAAAAAATCGAACGTTTTTTTACTCGTGGTTTGGACT  
 1200 TATGATTCAAAGTGTGTGIGGCTTGAGATCCTTATGGGTATATTCATTGCTGGCACCT  
 1260

Start

To Page



AGGGAGAGGCAGAAGAAGATAAGAGAGAACGAGAAAAGACACCGGTAAACTTTCTCGAA  
TGCTATATATACCACCTGIGGACTTCACCAAGACAATGGCTAGAGGATAGGGAGCAGAGA  
1501 -----+-----+-----+-----+-----+ 1560  
ACGATATATATGGTGGACACCTGAAGTGGTTCGTACCGATCTCCTATCCCTCGTCTCT  
ATGTTGCAAAATGGTAACATTTCAATGACTTAACTGTTTTGCTGCCAAGGTTCCTTATCC  
1561 -----+-----+-----+-----+-----+ 1620  
TACAACGTTTTACCATTGTAAAGTTACTGAATTGACAAAACGACGGTTCACCAACGAATAGG  
TATGAAAATTCAGCACATTAAAAGAGCTTATACATGCTCCCTAGAGTCAATACTCTTGCA  
1621 -----+-----+-----+-----+-----+ 1680  
ATACTTTTAAGTCGTGTAATTTTCTCGAATATGTACGAGGGATCTCAGTTATGAGAACGT  
TTTTCCCCCTCCTGCTCGGGGGGAAAAGGTTGACATTTCTGGCCCATTTCCCTTCTCAGC  
1681 -----+-----+-----+-----+-----+ 1740  
AAAAGGGGGAGGACGAGCCCCCTTTTTCCAACGTAAAGACCGGGTAAAGGAAGAGTCG  
TIGGTTTGTTTGAATTGATGCTTGIGGAATGGIATTTCACTTTAAGAGTGAAGATCC  
1741 -----+-----+-----+-----+-----+ 1800  
AACCAAACAACTTAACTACGAACACCTTACCATAAAGTAATGAAATTCTCACTTCTAGG  
  
HindIII  
|  
ATAGTGAAATTGGATGGATGGTTGAATTAGACGACCATTAAAGCTTGGATCCTCTAGAGCG  
1801 -----+-----+-----+-----+-----+ 1860  
TATCACTTTAACCTACCTACCAACTTAATCTGCTGGTAATTGAACTTAGGAGATCTCGC  
GCCGCCGACTAGTGAGCTCGTCGACCCGGGAATT  
1861 -----+-----+-----+-----+ 1894  
CGCGCGCTGATCACTCGAGCAGCTGGGCCCCTTAA



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27N

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rat clone

10 30 50  
GTCGACCCACGCGTCCGGGGTGTGTAGATATTTTTCCTTTGGAAGAAATACTGAGCACC  
70 90 110  
AAGGCTGAGATGACACTGAAGTATTTATGGCTGGTGGCCCTCGTGGCTCTATACATTTCA  
MetThrLeuLysTyrLeuTrpLeuValAlaLeuValAlaLeuTyrIleSer  
130 150 170  
CCCATCCAGTCTCAGAACTGTGTGTATCTGGATCATACCATCTTGGAACATGAACTT  
ProIleGlnSerGlnAsnCysValTyrLeuAspHisThrIleLeuGluAsnMetLysLeu  
190 210 230  
CTGAGCAGCATCAGGACCACCTTTCCCTTAAGATGTCATAAAGATATCACGGATTTTGAG  
LeuSerSerIleArgThrThrPheProLeuArgCysLeuLysAspIleThrAspPheGlu  
250 270 290  
TTTCTCAAGAGATTCTGCTGTACGTCCAGCATGTGAAAAAGGACATAAAGGCAGTCACC  
PheProGlnGluIleLeuLeuTyrValGlnHisValLysLysAspIleLysAlaValThr  
310 330 350  
TATCATATATCTTCTCTGGCGCTAATTATTTTTCAGTCTTAAAGACTCCATCTCCCTGGCG  
TyrHisIleSerSerLeuAlaLeuIleIlePheSerLeuLysAspSerIleSerLeuAla  
370 390 410  
ACAGAGGAACGCTTGGAACGTATCAGATCGGGACTTTTICAAACAAGTGCAGCAAGCTCGA  
ThrGluGluArgLeuGluArgIleArgSerGlyLeuPheLysGlnValGlnGlnAlaArg  
430 450 470  
GAGTGCATGGTAGACGAGGAGAACAAGAACACGGAGGAGCAGTACATCACAACTCTCT  
GluCysMetValAspGluGluAsnLysAsnThrGluGluAspSerThrSerGlnHisPro  
490 510 530  
CACTCAGAGGGCTTCAAGGCAGTCTACCTGGAATTGAACAAGTATTTCTTTCAGAATCAGA  
HisSerGluGlyPheLysAlaValTyrLeuGluLeuAsnLysTyrPhePheArgIleArg  
550 570 590  
AAGTTCCTGGTAAATAAGAAATACAGTTTCTGTGCTGGAAGATTGTCTGGTGGTGAATA  
LysPheLeuValAsnLysLysTyrSerPheCysAlaTrpLysIleValValValGluIle  
610 630 650  
AGAAGATGTTTCAGTATATTTTACAACTACTCAACATGAATTGAGAATCATCCAGCTTC  
ArgArgCysPheSerIlePheTyrLysLeuLeuAsnMetAsnEnd  
670 690 710  
AAGCAAGAACTTAGATAGAAGTTGTGACTGCTCAAATGTCCCAAGAACGCTTGATTCTA  
730 750 770  
AGGCTATTGGGAGTCTGCTGCTACACACTTCGGACGCAAGACTTTTCAAGGTCAGGGTTC  
790 810 830  
AAGGCAGTACAGTCAAAGGAAGTCTTATGTTAAGCAAAAGAAAAATTTTCAGTGGAAAAGC  
850 870 890  
TAGCAGAAATGTCAACTGTCAAAAAACAACCTTATGGATTATGGCATTGACGTTACTAG  
910 930 950  
CAAAAAAATAAAACAAAAAACAACAGTCACTAAAAAAGGGCGGC

CGC

# Nucleotide comparison

WChen

DNA=hgmrep3-6.2

assembled by JK

9808903.com

JFN

to: Mrpe3-00078-F6-Wz.Ctg check: 4485 from: 1 to: 963

From: INTRON::DONGYINY 16:07:43.44

To: WCHEN

CC:

Subj:

From: INTRON::JCAO 12:24:38.19

To: DONGYINY . . .

Symbol comparison table: Gencoredisk:[Gcgcore.Data.Rundata]Swgapdna.Cmp  
CompCheck: 2335

Gap Weight: 50 Average Match: 10.000  
Length Weight: 3 Average Mismatch: -9.000

Quality: 1457 Length: 634  
Ratio: 2.392 Gaps: 6  
Percent Similarity: 63.516 Percent Identity: 63.516

Match display thresholds for the alignment(s):

| = IDENTITY

: = 5

, = 1

9808903.Rev x Mrpe3-00078-F6-Wz.Ctg 15:04 ..

555 TCTTCTGGATTTTTTTAGCTT..GCAAAAAAATGAGCACCAACCTGATA 602

| | | | | | | | | | | | | | | | | | | | | |

21 TGTTGTAGATATTTTTCCTTTGGAAGAAATACTGAGCACCAAGGCTGAGA 70

603 TGATTCAAAAGIGTTTGGGCTTGAGATCCTTATGGGTATATTTCATTGCT 652

| | | | | | | | | | | | | | | | | | | | | |

71 TGACACTGAAGTATTTATGGCTGGTGGCCCTCGTGGCTCTATACATTICA 120

653 GGCACCCCTATCCCTGGACTGTAACTTACTGAACGTTCAACCTGAGAAGAGT 702

| | | | | | | | | | | | | | | | | | | | | |

121 CCCATCCAGTCTCAGAACTGT.....GTGTATCTGGATCATAC 158

703 CACCTGGCAAAATCTGAGACATCTGAGTAGTATGAGCAATTCATTTCCCTG 752

| | | | | | | | | | | | | | | | | | | | | |

159 CATCTTGGAAAACATGAACTTCTGAGCAGCATCAGGACCACCTTTCCCT 208

753 TAGAATGICTACGAGAAAACATAGCTTTTGAGTTGCCCAAGAGTTTCTG 802

| | | | | | | | | | | | | | | | | | | | | |

209 TAAGATGICTAAAAGATATCAGGATTTTGAGTTTCCCTCAAGAGATTCTG 258

803 CAATACACCCAACCTATGAAGAGGGACATCAAGAAGGCCTTCTATGAAAT 852

| | | | | | | | | | | | | | | | | | | | | |

259 CTGTACGTCAGCATGTGAAAAAGGACATAAAGGCAGTCACCTATCATAT 308

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

TITLE \_\_\_\_\_

ZFN

853 GTCCCTACAGGCGCTTCAACATCTTCAGCC...AACACACCTTCAAATATT 899  
|| | ||| | | || |||| | || | || |  
309 ATCTTCTCTGGCGCTAATTATTTTCAGTCTTAAAGACTCCATCTCCCTGG 358  
900 GGAAAGAGAGACACCTCAAACAAATCCAAATAGGACTTGATCAGCAAGCA 949  
|| |||| | | | ||| | |||| | ||||  
359 CGACAGAGGAACGCTTGAACGTATCAGATCGGGACTTTTICAAACAAGTG 408  
950 GAGTACCTGAACCAATGCTTGGAGGAAGACGAGAATGAAAATGAAGACAT 999  
|| | | ||| ||| || || |||| | | |||  
409 CAGCAAGCTCGAGAGTGCATGGTAGACGAGGAGAACAAGA.....ACAC 452  
1000 GAAAGAAATGAAAGAGAATGAGATGAAACCCCTCAGAAGCCAG.GGTCCCC 1048  
| | | | | | | | | | | | | | | | | |  
453 GGAGG.....AGGACAGTACATCACAACATCCTCACTCAGAGGGCTTC 495  
1049 CAGCTGAGCAGCCTGGAAC TGAGGAGATATTTCCACAGGATAGACAATTT 1098  
|| | ||||| ||| | ||||| ||| || || ||  
496 AAGGCAGTCTACCTGGAATTGAACAAGTATTTCTTCAGAATCAGAAAGTT 545  
1099 CCTGAAAGAAAAGAAATACAGTGACTGTGCCTGGGAGATTGTCCGAGTGG 1148  
|||| | | ||||| ||||| ||||| ||||| |||||  
546 CCTGGTAAATAAGAAATACAGTTTCTGTGCCTGGAAGATTGTGGTGGTGG 595  
1149 AAATCAGAAGATGTTTGTATTACTTTTACAAATT 1182  
|||| ||||| |||| | ||||| ||||  
596 AAATAAGAAGATGTTTCAGTATATTTTACAACT 629



ITN

Project No. \_\_\_\_\_

Book No. \_\_\_\_\_

protein sequence comp

BESTFIT of: 9808903.Pep check: 4904 from: 1 to: 201

TRANSLATE of: 9808903.rev check: 8672 from: 602 to: 1205  
generated symbols 1 to: 201.

REVERSE-COMPLEMENT of: 9808903.Con check: 57 from: 1 to: 1894  
When  
DNA-hgmrep3-6.2  
assembled by JK: [REDACTED] . . .

to: Mrpe3-00078-F6-Wz.Pep check: 5990 from: 1 to: 192

TRANSLATE of: mrpe3-00078-f6-wz.ctg check: 4485 from: 70 to: 646  
generated symbols 1 to: 192.

From: INTRON::DONGYINY [REDACTED] 16:07:43.44

To: WCHEN

CC:

Subj: . . .

Symbol comparison table: Gencoredisk: [Gogcore.Data.Rundata]Blosum62.Cmp  
CompCheck: 6430

Gap Weight: 12 Average Match: 2.912  
Length Weight: 4 Average Mismatch: -2.003

Quality: 292 Length: 194  
Ratio: 1.570 Gaps: 3  
Percent Similarity: 49.730 Percent Identity: 40.541

Match display thresholds for the alignment(s):

| = IDENTITY

: = 2

. = 1

9808903.Pep x Mrpe3-00078-F6-Wz.Pep [REDACTED] 14:55 ...

1 MIQKCLWLEILMGIFIAGTILSLDCNLLNVHLRRVTWQNLRLSSMSNSFP 50

| | | | | . : : | . | . | : : | : : | | | . . | |

1 MILKYLWLVALVALYISPIQSONC....VYLDHTLENMKLLSSIRITFP 46

51 VECLRENTAFELPQEFLOYTOPMKRDIKAFYEMSLQAFNIFS.QHIFKY 99

. | : : | | | | | . : : | | . | | | . .

47 LRCLKDITDFEFQEIILLYVQHVKKDIKAVTYHISSLALIIFSLKDSISL 96

100 WKERHLKQIQIGLDQQAIEYLNOCLEEDENENEDMKEMKENEMKPSEARVP 149

| | . . | | . | : : : : | | : : . .

97 ATEERLERIRSGLFKQVQQAECMVDEENKNTIEDSTSQHPHSEGFKAV. 145

150 QLSSLELRRYFHRIDNFLKEKKYSDCAWEIVRVEIRRLYYFYK 193

| | | : | | | | | | | | | | | | | | | |

146 ...YLELNKYFFRIRKFLVNKKYSFCWKIVVVEIRRCFSIFYK 186

To Page No. \_\_\_\_\_

nessed & Understood by me,

Date

Invented by

7

Date

43  
36  
50

50

85  
86  
100

100

122  
121  
150

150

157  
170  
200

200

187  
203  
233

231

17% idem

13  
4  
2

4.

30  
50

50

82  
85  
84

85

86  
100

100

118  
122  
121

122

150

150

163  
157  
170

157

170  
200

200

191  
187  
201

191

201  
231

231

IFN

### Display Expanded Sequence

From Page No. \_\_\_\_\_

**Chen, Wen**

From: Schultz, Henry  
Sent: Friday, [REDACTED] 5:15 PM  
To: Chen, Wen  
Subject: RE: human interferon like seq.

Wen - the human predicts cytokine strongly  
scoreaaccomp= -2.9 scoredipep= 19 ACCEPT (Probability 91%).

The human is predicted to be signal peptide as follows:

MIQKCLWLEILMGIFIAGTL; cleavage LD.....etc

For the rat, the cytokine prediction is lost but the signal peptide is:

MTLKYLWLVALVALYISPIQS cleavage QN....etc

Henry

-----  
From: Chen, Wen  
Sent: Friday, [REDACTED] 4:02 PM  
To: Schultz, Henry  
Subject: human interferon like seq.

Henry: please help me look at the signal peptide for the human clone sequence.  
Thanks.

Wen Chen

Human sequence:

MIQKCLWLEILMGIFIAGTL SLDCNLLNVH LRRVTWQNLRL HLSSMSNSFP

51 VECLRENIAF ELPQEFLQYT QPMKRDIKKA FYEMSLQAFN IFSQHTFKYW

101 KERHLKQIQI GLDQQAAYLN QCLEEDENEN EDMKEMKENE MKPSEARVPQ

151 LSSLELRRYF HRIDNFLKEK KYSDCAWEIV RVEIRRCLYY FYKFTALFRR

201 K



From Page No. \_\_\_\_\_

gLysEnd  
 1270 1290 1310  
 TCCTCCTCCATCTTCTTTTAAGGATTGTTGCTGCTGCTGTAAGCCTGTCCTCAGTTGG  
 1330 1350 1370  
 ACTGGTAGCCTCGGAACATCAGGGACACTCACCTCTCTAAGGAGAGGTAATGCCAACCAT  
 1390 1410 1430  
 CCTCAGGGTGACCAAGAGTCTCTTAGAAAGTCTTTAAGACATTTTAAAGGAATAAGAT  
 1450 1470 1490  
 TCCCTCTCCGCTCTTCTTCTATCTCTCTGCTCTTTTCTGTTGGCCATTTTGAAAGAGCTT  
 1510 1530 1550  
 TGCTATATATACCACTGTGGACTTCACCAAGACAATGGCTAGAGGATAGGGAGCAGAGA  
 1570 1590 1610  
 ATGTTGCAAAATGGTAACATTTCAATGACTTAACTGTTTGTCTGCCAAGGTTCCTTATCC  
 1630 1650 1670  
 TATGAAAATTTCAGCACATTAAAGAGCTTATACATGCTCCCTAGAGTCAATACTCTTGCA  
 1690 1710 1730  
 TTTTCCCCCTCCGCTCGGGGGGAAAAGGTTGACATTTCTGGCCATTTTCTTCTCAGC  
 1750 1770 1790  
 TTGGTTTGTGTTGAATGATGCTTGTGGAATGGTATTTCAATTACTTTAAGAGTGAAGATCC  
 1810 1830 1850  
 ATAGTGAAATTGGATGGATGGTTGAATTAGACGACCATTAAAGCTTGGATCCTCTAGAGCG  
 1870 1890  
 GCCGCCGACTAGTGAGCTCGTCCGACCCGGCAATT

0 400 800 1200 1600 2000

## ORF Map (Universal)

Frame 3

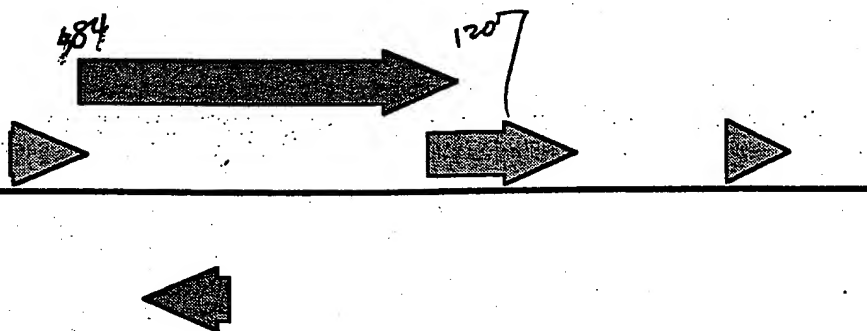
Frame 2

Frame 1

Frame -1

Frame -2

Frame -3



One more ATG @ 584 in frame with ATG starting from 602.

To Page 1

Witnessed &amp; Understood by me.

Date

Invented by

Date



# RESEARCH SUMMARY PAGE

01067  
McDonnell  
GN-0001

## Gene Name:

## All Known Alias Gene Names:

Human: Zhwxc00-00001-a1  
Rat: Agp-22423-a1

Member of the interferon family of proteins  
Name: Interferon-like protein.

## Investigator(s):

## Initial Date of Summary Preparation:

Duanzhi Wen, Andrew Welcher, Michael  
Kelley

Initial invention disclosure filed [REDACTED]  
This summary filled in on [REDACTED]

## Description of Project:

This novel member of the interferon family of proteins is related to the beta, alpha, and omega subfamilies. As an interferon it would be expected to have anti-infective and anti-proliferative uses. Additionally, it might find use in the treatment of multiple sclerosis and other pathologies requiring immunomodulation.

## Gene Nucleotide Sequence:

### Human:

```
1  CGCGTACGTA AGCTTAATTT AACAAAATTG GAAAAACCTA AACTATACTG
51  TGCTCTGGTG ACCTAGCAAT CAAATAATCA CAGTCATTTG GTCAATGTCT
101 ATGATTAAC TCAATGAGACA GGATGTTTGG CTATAGCACC AGGTACAAAA
151 AATATATTTT CATGAAGGAT CACTCCCTCT TATGTAATAG ATTTGGGTGA
201 GTGAGTGAGT GAGTGAGTGC ATGGACTCAC AGCTTTTGGC TTTCTGAAAT
251 ACCCTGCATC AGTCTTGTTA TGATGATTCC TTAGTGCTGG GATGGATCAT
301 CCAGGCATTT AAGGTAACAC GATGGTAATT CTTTGCTCAT TTTTCAGGGA
351 AAAAAAAG TTATCACTTC CAAAGTCGGC ATAGTCACCC GAAGTAAAAA
401 AAAAAAAGC AAAAAAAGC CTCAGAGGCA AAGGAAAGGG GCCGCAACCT
451 TGGTAACTG TGAAATGACG AATGAGAAAA CTCCTCCTGC TGAAGATATT
501 CAGGTATATA AAGGCACATG AAGGAAACT CAAAACATCA TTGTCATATA
551 CACATCTTCT GGATTTTTTA GCTTGCAAAA AAAATGAGCA CCAAACCTGA
601 TATGATTCAA AAGTGTGTTG GGCTTGAGAT CCTTATGGGT ATATTCAATTG
651 CTGGCACCTT ATCCCTGGAC TGTAACCTAC TGAACGTTCA CCTGAGAAGA
701 GTCACCTGGC AAAATCTGAG ACATCTGAGT AGTATGAGCA ATTCATTTC
751 TGTAGAATGT CTACGAGAAA ACATAGCTTT TGAGTTGCCC CAAGAGTTTC
801 TGCAATACAC CCAACCTATG AAGAGGGACA TCAAGAAGGC CTTCTATGAA
851 ATGTCCTTAC AGGCCTTCAA CATCTTCAGC CAACACACCT TCAAATATTG
901 GAAAGAGAGA CACCTCAAAC AAATCCAAAT AGGACTTGAT CAGCAAGCAG
951 AGTACCTGAA CCAATGCTTG GAGGAAGACG AGAATGAAAA TGAAGACATG
1001 AAAGAAATGA AAGAGAATGA GATGAAACCC TCAGAAGCCA GGGTCCCCCA
1051 GCTGAGCAGC CTGGAACCTG GGAGATATTT CCACAGGATA GACAATTTCC
1101 TGAAGAGAAA GAAATACAGT GACTGTGCTT GGGAGATTGT CCGAGTGGAA
1151 ATCAGAAGAT GTTTGTATTA CTTTACAAA TTTACAGCTC TATTCAGGAG
1201 GAAATAAGGT ATATTTTTGG AATTAAATTT CCTTTTCCCT CCGAAATCTC
1251 TTTCTCCTTC TCCTCCTCCA TCTTCTTTT AAGGATTGTT GTGCTGTCTC
1301 GTAAGCCTGT CCTCAGTTGG ACTGGTAGCC TCGGAACATC AGGGACACTC
```

```

1351 ACCTCTCTAA GGAGAGGTAA TGCCAACCAT CCTCAGGGTG ACCAAGAGTC
1401 TCCTTAGAAA GTCTTTAAGA CATTTTTAAA GGAATAAGAT TCCCTCTCCG
1451 TCTTCTCTA TTCTCTCTTG CTCTTTTCTG TGGCCATTTT GAAAGAGCTT
1501 TGCTATATAT ACCACCTGTG GACTTCACCA AGACAATGGC TAGAGGATAG
1551 GGAGCAGAGA ATGTTGCAAA ATGGTAACAT TTCAATGACT TAACTGTTTT
1601 GCTGCCAAGG TTGCTTATCC TATGAAAATT CAGCACATTA AAAGAGCTTA
1651 TACATGCTCC CTAGAGTCAA TACTCTTGCA TTTTCCCCCT CCTGCTCGGG
1701 GGGAAAAAGG TTGACATTTT TGGCCCATTT CCTTCTCAGC TTGGTTTGTT
1751 TGAATTGATG CTTGTGGAAT GGTATTTTCA TACTTTAAGA GTGAAGATCC
1801 ATAGTGAAAT TGGATGGATG GTTGAATTAG ACGACCATTA AGCTTGGATC
1851 CTCTAGAGCG GCCGCCGACT AGTGAGCTCG TCGACCCGGG AATT

```

Rat:

```

1 GGGTGTGTA GATATTTTTC CTTTGAAGA AATACTGAGC ACCAAGGCTG
51 AGATGACACT GAAGTATTTA TGGCTGGTGG CCCTCGTGGC TCTATACATT
101 TCACCCATCC AGTCTCAGAA CTGTGTGTAT CTGGATCATA CCATCTTGGA
151 AAACATGAAA CTTCTGAGCA GCATCAGGAC CACCTTTCCC TTAAGATGTC
201 TAAAAGATAT CACGGATTTT GAGTTTCCTC AAGAGATTCT GCTGTACGTC
251 CAGCATGTGA AAAAGGACAT AAAGGCAGTC ACCTATCATA TATCTTCTCT
301 GGCGCTAATT ATTTTCAGTC TTAAAGACTC CATCTCCCTG GCGACAGAGG
351 AACGCTTGGA ACGTATCAGA TCGGGACTTT TCAAACAAGT GCAGCAAGCT
401 CGAGAGTGCA TGGTAGACGA GGAGAACAAG AACACGGAGG AGGACAGTAC
451 ATCACAACAT CCTCACTCAG AGGGCTTCAA GGCAGTCTAC CTGGAATTGA
501 ACAAGTATTT CTTCAGAATC AGAAAGTTCC TGGTAAATAA GAAATACAGT
551 TTCTGTGCCT GGAAGATTGT CGTGGTGGAA ATAAGAAGAT GTTTCAGTAT
601 ATTTTACAAA CTACTCAACA TGAATTGAGA ATCATCCAGC TTCAAGCAAG
651 AACTTAGATA GAAGTTGTGA CTGCTCAAAT GTCCCCAAGA ACGCTTGATT
701 CTAAGGCTAT TGCGAGTCTG CTGCTACACA CTTCCGACGC AAGACTTTTC
751 AAGGTCAGGG TTCAAGGTAG TACAGTCAAA GGAAGTCTTA TGTTAAGCAA
801 AAGAAAAATT TCAGTGGAAG AGCTAGCAGA AATGTCAACT TGTCAAAAAA
851 ACAACTTATG GATTATGGCA TTGACGTTAC TAGCAAAAAA AATAAAACAA
901 AAAAAAACAA AAA

```

### Gene Amino Acid Sequence:

Human:

```

1 MSTKPDMIQK CLWLEILMGI FIAGTSLSDC NLLNVHLRRV TWQNLRLHLS
51 MSNSFPVECL RENIAFELPQ EFLQYTQPMK RDIKKAFYEM SLQAFNIFSQ
101 HTFKYWKERH LKQIQIGLDQ QAEYLNQCLE EDENENEDMK EMKENEMKPS
151 EARVPQLSSL ELRRYPHRID NFLKEKKYSYD CAWEIVRVEI RRCLYYPYKF
201 TALFRRK*

```

Rat:

```

1 MTLKYLWLV LVALYISPIQ SQNCVYLDHT ILENMKLLSS IRTTFPLRCL
51 KDITDFEFPQ EILLYVQHV KDIKAVTYHI SSLALIIFSL KDSISLATEE
101 RLERIRSLGF KQVQQAECM VDEENKNTTE DSTSQHPHSE GFKAVYLELN
151 KYFFRIRKFL VNKKYSFCAW KIVVVEIRRC FSIFYKLINM N*

```

### Figure Containing cDNA and Amino Acid Sequences:

Human:



## Sequence Analysis of Human IFN-novel

[illegible]

A human gene which encodes a novel protein of 207 amino acids was isolated by screening the human genomic DNA library using a rat cDNA clone. The deduced amino acid sequence of this novel gene is indicated below the first nucleotide of each codon, and the termination codon is marked with an asterisk. The protein starts with cysteine, and the signal peptide is underlined. This novel protein is 27% identical to human IFN- $\beta$ .

**Rat:**

1	GGGTGTGTAGATATTTTCTCTTGGGAAGAAATACTGAGCACCAAGGCTGAGATGACACT	60
1	— M T L	3
61	GAAGTATTTATGGCTGGTGGCCCTCGTGGCTCTATACATTTACCCATCCAGTCTCAGAA	120
4	K Y L W L V A L V A L Y I S P I O S O N	23
121	CTGTGTGTATCTGGATCATAACCATCTTGGAAAACATGAAACTTCTGAGCAGCATCAGGAC	180
24	C V Y L D H T I L E N M K L L S S I R T	43
181	CACCTTTCCCTTAAGATGTCTAAAGATATCACGGATTTGAGTTTCTCTCAAGAGATTCT	240
44	T F P L R C L K D I T D F E F P Q E I L	63
241	GCTGTACGTCCAGCATGTGAAAAAGGACATAAAGGCAGTCACCTATCATATATCTTCTCT	300
64	L Y V Q H V K K D I R A V T Y H I S S L	83
301	GGCGCTAATTATTTTCAGTCTTAAAGACTCCATCTCCCTGGCGACAGAGGAACGCTTGGAA	360
84	A L I I F S L K D S I S L A T E E R L E	103
361	ACGTATCAGATCGGGACTTTTCAACAAGTGCAGCAAGCTCGAGAGTGCATGGTAGACGA	420
104	R I R S G L F K Q V Q Q A R E C M V D E	123
421	GGAGAACAAGAACACGGAGGAGGACAGTACATCACAACATCCTCACTCAGAGGGCTTCAA	480
124	E N K N T E E D S T S Q H P H S E G F K	143
481	GGCAGTCTACCTGGAAATTGAACAAGTATTTCTTCAGAATCAGAAAGTTCTGGTAAATAA	540
144	A V Y L E L N K Y F F R I R K F L V N K	163
541	GAAATACAGTTTCTGTGCCTGGAAGATTGTCGTGCTGGAATAAGAAGATGTTTCAGTAT	600
164	K Y S F C A W K I V V V E I R R C F S I	183
601	ATTTTACAACTACTCAACATGAATTGAGAATCATCCAGCTTCAAGCAAGAACTTAGATA	660
184	F Y K L L N N N *	192
661	GAAGTTGTGACTGCTCAAATGTCCCAAGAAGCGCTTGATTCTAAGGCTATTGCGAGTCTG	720
721	CTGCTACACACTTCGGACGCAAGACTTTTCAAGGTCAAGGTTCAAGGCAGTACAGTCAAA	780
781	CGAAGTCTTATGTTAAGCAAAAGAAAAATTTCAAGTGGAAAAGCTAGCAGAAATGTCAACT	840
841	TGTCAAAAAACAACCTTATGCGATTGCGATTGACGTTACTAGCAAAAAAATAAAACAA	900
901	AAAAAACAACAGTCACTAAAAA	923

### Cloning Information:

The rat sequence was cloned from a rat placenta cDNA library as part of an EST project and was identified by computer analysis as being a novel member of the interferon family of proteins. Briefly, rat embryo day 17 [E17] placenta mRNA was isolated by standard methods (unnecessary information) (Chomczynski, P. and Sacchi, N., Anal. Biochem. 162, 156, 1987). cDNA was synthesized using the SuperScript Plasmid cDNA kit supplied by GIBCO/BRL and subcloned into the pSPORT1 (GIBCO/BRL) vector into the Sal I and Not I restriction sites.

### Cloning of Human IFN-like gene:

Multiple attempts to clone the human IFN-like gene from a variety of human tissue cDNA libraries failed to yield positive clones. However, a human tissue Northern Blot

hybridized with a PCR-generated radioactive rat probe revealed an 1.8 kb Hind III fragment in certain batches of human pancreas mRNA. Attempts to clone this corresponding message in a pancreas cDNA library failed to recover any positive clones.

Examination of the genomic structures of known IFNs revealed that IFN, especially the members in the IFN $\alpha$  family, all share a unique intronless genomic structure. Therefore, screening of human genomic DNA might yield the complete human IFN-like gene. We started with  $1 \times 10^6$  human lambda genomic clones (Stratagene, Cat. No. 946206) for primary screening at a density of 50,000 clones / plate (*unnecessary information*). Nitrocellulose filters (*unnecessary information*) (S&S) were prepared by standard techniques (Molecular Cloning, A Laboratory Manual, Sambrook, Fritsch, and Maniatis editors).

The following conditions were used.

- Prehybridization and hybridization conditions: 30% formamide, 5x SSC, 2x Denhart's, 10 $\mu$ g/ml Salmon sperm DNA, 0.2% SDS, 2mM EDTA and 0.1% pyrophosphate. Hybridization was conducted overnight at 42°C. The washings were done under following conditions: 1x SSC, 0.1%SDS at room temperature for 30-60 minutes followed by 0.2x SSC and 0.1%SDS at 55°C for 15 minutes.
- Generation of radioactive PCR probe (*unnecessary information*): rat cDNA full-length fragment 20ng, primer 1795-01 and 1795-02, 20 pmol each, 1mmol dNTP (dCTP @ 0.01mmol), 32P-dCTP 5 ml and 4mM MgCl<sub>2</sub>. Reaction condition: denature at 94°C for 30sec, anneal at 60°C for 30sec and elongate at 72°C for 1 minute. The reaction is repeated for a total of 45 times. Simultaneously a "cold" PCR reaction is performed under exact condition except the dNTP mix is dCTP balanced. The radioactive probe was purified by Quick Spin G-50 column and boiled at 100°C for 10 minutes before chilling on dry ice for 20 minutes. The probe is usually  $5 \times 10^5$  cpm/ $\mu$ l.

Three positive clones were recovered after primary, secondary screening and subsequently purified to homogeneity. The lambda phage DNA was prepared by a solid plate culture method. The NotI insert from these clones were excised out and ligated into pSport (GIBCO BRL) vector and transformed into DH10 E. coli strain. The transformants were prepared by Qiagen Spin Column plasmid prep kit. The plasmid DNA was then digested with HindIII. The digested fragments were resolved on agarose gel and transferred to a nylon membrane for Southern Blot analysis. The analysis was conducted under the same condition genomic screening was carried out. The corresponding fragment recognized by "hot" rat probe was then subcloned in pSport vector for sequencing analysis. According to the HindIII digestion pattern, we determined these three independent clones were likely to contain identical genomic insert. The sequencing analysis confirmed our speculation. This 1.8kb HindIII fragment contains an open reading frame of 624 base pairs that has 64% similarity to the sequence of rat mrpe3-00078-F6-Wz. In terms of similarity in amino acid sequence, the human sequence is 40.5% identical to and 50% similar to that of rat. All 5 predicted Cysteine residues were perfectly aligned with those in rat protein sequence. Moreover, the human sequence is predicted to contain a signal peptide and cleavage site. The human IFN-like protein is strongly predicted to resemble a secreted cytokine molecule (91% probability).

## Homology of Multiple Gene Family Members:

### Amino Acid Sequence Alignment of Human IFN-novel, Rat IFN-novel and Human IFN- $\beta$

Human IFN-novel	QOK...I LMOI...CTL SL...-----DCNL LNVELARVTN	36
Human IFN-beta	QOK...I LMOI...CTL SL...-----DCNL LNVELARVTN	43
Rat IFN-novel	QOK...I LMOI...CTL SL...-----DCNL LNVELARVTN	12
Consensus	QOK...I LMOI...CTL SL...-----DCNL LNVELARVTN	60
Human IFN-novel	QNLHLSMS NEPPVECLRE NY...LQV...K...QAFYEM	86
Human IFN-beta	QNLHLSMS NEPPVECLRE NY...LQV...K...QAFYEM	85
Rat IFN-novel	QNLHLSMS NEPPVECLRE NY...LQV...K...QAFYEM	82
Consensus	QNLHLSMS NEPPVECLRE NY...LQV...K...QAFYEM	100
Human IFN-novel	QAFNI...T PKY-WKE...-----RHLEKIQIG LDQQAETL	121
Human IFN-beta	QAFNI...T PKY-WKE...-----RHLEKIQIG LDQQAETL	122
Rat IFN-novel	QAFNI...T PKY-WKE...-----RHLEKIQIG LDQQAETL	118
Consensus	QAFNI...T PKY-WKE...-----RHLEKIQIG LDQQAETL	150
Human IFN-novel	CLEEDSENE DMCKCKENEM KP...ARVPO LSSLEK...YF HRIDNVLK	170
Human IFN-beta	CLEEDSENE DMCKCKENEM KP...ARVPO LSSLEK...YF HRIDNVLK	157
Rat IFN-novel	CLEEDSENE DMCKCKENEM KP...ARVPO LSSLEK...YF HRIDNVLK	163
Consensus	CLEEDSENE DMCKCKENEM KP...ARVPO LSSLEK...YF HRIDNVLK	200
Human IFN-novel	K...PTALPRA K	201
Human IFN-beta	K...PTALPRA K	187
Rat IFN-novel	K...PTALPRA K	191
Consensus	K...PTALPRA K	231

- Human IFN-novel is most close to human IFN- $\beta$ , with 30% identity. Four out of five cysteine residues are conserved between them.

## Presence and Distribution of mRNA in Different Tissues:

Northern blot analysis detected IFN-like mRNA in several different stages of mouse and rat embryos. Northern blots used RNA isolated as above. The full-length rat cDNA was used as a probe. Prehyb conditions were 40 % formamide, 5X SSC, 1 mM EDTA, 0.1 % SDS, for 4 h at 42°C. Hyb conditions were the same as above except were done overnight at 42°C. Blots were washed with 0.2x SSC, 1 mM EDTA, and 0.1% SDS for 30 min at 60°C.

RT-PCR (*conditions are not necessary - standard technology*) identified IFN-like mRNA in the following human tissues : pancreas, small intestine, prostate, uterus, thyroid, and placenta.

## Recombinant Protein Expression:

Production of human and rat IFN-like protein in E. coli :

Waiting on data from Karen Sitney. However, the E. coli protein did not appear to be folded correctly and has not yet generated any biologically active material.

## **Production of human and rat IFN-like protein in a mammalian expression system:**

Several versions of the human and rat IFN-like protein have been produced in a mammalian expression system (either CHO or 293 cells). The proteins synthesized were either the native protein itself, or a native protein-Fc fusion. Some of the Fc fusion constructs contained a cleavage site which allows the native protein to be released from the Fc portion after being produced in the conditioned media of CHO cells.

PCR amplification of IFN-like molecule:

PCR primers were selected to amplify the coding sequence of rat/human IFN-like molecule:

### Rat IFN-Like Molecule primers:

- IFN-Like molecule Fc-fusion:

1847-77 CCC AAG CTT ACC ATG ACA CTG AAG TAT TTA TG

Forward primer: Hind III site plus ATG

1847-78 AAG GAA AAA AGC GGC CGC ATT CAT GTT GAG TAG

- Reverse primer: Not I site and no stop codon for Fc fusion

Soluble IFN-like molecule:

1896-56 ACG CGT CGA CTC ATC AAT TCA TGT TGA GTA GTT TG

Reverse primer: Sal I site plus 2 stop codons (for pDSR $\alpha$  cloning).

1896-57 AAG GAA AAA AGC GGC CGC TCA TCA ATT CAT GTT GAG TAG

Reverse primer: Not I site plus two stop codons (for pCEP4 cloning).

### Human IFN-like primers:

- Soluble human IFN-like primers:

1954-45 ACG CGT CGA CTT ATT ATT TCC TCC TGA ATA G

Reverse primer: Sal I site plus 2 stop codons (for pDSR $\alpha$  cloning).

1954-46 AAG GAA AAA AGC GGC CGC TTA TTA TTT CCT CCT GAA TAG AGC

Reverse primer: Not I site plus two stop codons (for pCEP4 cloning).

- Human IFN like-Fc fusion primers:

1955-44 CCC AAG CTT ACC ATG AGC ACC AAA CCT GAT ATG

Forward primer: Hind III site with 1st ATG

1954-47 CCC AAG CTT ACC ATG ATT CAA AAG TGT TTG TGG C

Forward primer: Hind III site with 2nd ATG

1954-48 AAG GAA AAA AGC GGC CGC GCG GCC CTC GAT TTT CCT CCT GAA TAG AGC TGT AA

Reverse primer: Not I site, no stop codon with *Factor Xa* cleavage site and Fc fusion

1954-49 AAG GAA AAA AGC GGC CGC TTT CCT CCT GAA TAG AGC TGT AA

Reverse primer: Not I site and no stop codon for Fc fusion

PCR Reaction:

### Rat:

*Reaction Mixture:* template 20 ng, 1847-77 and 1847-88 or 1896-56/57, 20 pmol each, 1mmol dNTPs, 4mM MgCl<sub>2</sub>, 1X PCR buffer, 5u Taq polymerase.

*Reaction condition:* 2 cycle-linked PCR.

94 °C for 30 °C sec, 50 °C for 30 sec and 72 °C for 1 minute, for a total of 4 cycles, follow by 94

°C for 30 °C sec, 55 °C for 30 sec and 72 °C for 1 minute, for a total of 26 cycles.

**Human interferon-like protein** PCR conditions:

**Reaction Mixture:** template 20 ng, 1955-44 and 1954-45 or 1954-46 (soluble form) or 1945-48/49 (Fc fusion), 20 pmol each, 1mmol dNTPs, 4mM MgCl<sub>2</sub>, 1X PCR buffer, 5u Taq polymerase.

**Reaction condition:** 2 cycle-linked PCR.

94 °C for 30 °C sec, 48 °C for 30 sec and 72 °C for 1 minute, for a total of 4 cycles, follow by 94 °C for 30 °C sec, 55 °C for 30 sec and 72 °C for 1 minute, for a total of 26 cycles.

While 1955-44 primer generates an ORF using first Met in the coding region, a separate PCR with 1954-47 to obtain an insert using 2nd downstream Met was also generated. But in terms of secretion efficiency, when tested in 293 EBNA transient transfection, there was no detectable difference could be defined.

For both rat and human, the PCR products were purified by Qiagen PCR purification spin column and subjected to restriction digestion by respective enzymes (HindIII and NotI (pCEP4) or SalI(pDSRα)). After digestion, the fragment was purified from agarose gel with Qiagen gel purification spin column. The purified fragment was quantified and ligated into pCEP4 (for native form), pCEP4-Fc (for Fc form) or pDSRα (native form or Fc form) vectors respectively. The ligation was transformed into DH10. The transformants were picked for miniprep and subsequent sequencing verification. Accuracy of each cloning fragment was verified by sequencing including the Fc junction sequence. The clone was then maxi-prepared for tissue culture transfection experiments. The IFN-Fc fragment in pCEP4-Fc vector can be released by cutting this vector with HindIII and SalI and re-ligated this fragment into pre-digested pDSRα to yield a vector suitable to transfect CHOD<sup>-</sup> cells.

**Transfection:**

- Protocol for transfection into 293 EBNA and CHO cells with lipofectin was adopted from the one used by Jin Cao. Same protocol was used to generate both transient and stable transfectants.
- A commercial available calcium phosphate transfection kit was used in CHO cell stable transfection (protocol is attached).
- A CHO cell transfection and selection protocol from Yi Luo was utilized, except calcium phosphate transfection procedure, which has a commercially available kit.

In general, lipofectin transfection yields more stable transfection colonies. Those colonies express comparable level of secreted proteins as those picked from calcium phosphate method.

**Generate conditioned media containing recombinant protein.**

In order to conduct functional studies on this interferon-like molecule, large quantity of conditioned media (CM) were generated from a pool of hygromycin selected 293 EBNA clones. The cells were cultured in Nunc Triple Flask (500cm) to 80% confluence before switching to serum free media for a week before harvesting. The CM was then sent to purification with protein A affinity chromatography. The purified protein was then used to generate a rabbit polyclonal antibody and to test for in vitro activities. The processing of signal peptide as well as partial amino acid sequence was verified by peptide sequencing.

### **Purification of human IFN-like-Fc**

Conditioned media from CHO cells expressing huIFLM-Fc was thawed and 0.2µm filtered. The filtered material was loaded onto a Protein G column that was previously equilibrated with PBS, pH 7.0. After loading, the column was washed with PBS until the absorbance at A<sub>280</sub> reached baseline. The protein was eluted from the column with 0.1M Glycine-HCl pH 2.7 and immediately neutralized with 1M Tris-HCl pH 8.5. Fractions containing huIFLM-Fc were pooled and dialyzed into PBS and stored at -70°C.

### **Factor Xa cleavage of human IFN-like-Fc**

The human IFN-like-Fc construct has a Factor Xa cleavage site (IEGR) inserted between the Fc and huIFLM. This site is cleaved with restriction protease factor Xa. The human IFN-like-Fc in PBS was dialyzed into 50mM Tris-HCl, 100mM NaCl, 2mM CaCl<sub>2</sub>, pH 8.0. The Factor Xa was added to the dialyzed protein at 1/100 (w/w). The sample was incubated overnight at room temperature.

### **Abs (available, ordered, proposed):**

#### **1. Polyclonal:**

Polyclonal antibodies were prepared using both rat and human proteins produced in E. coli and CHO cells (from above) using standard immunological techniques. Antisera were positive for the proteins as determined by Western blot analysis (standard techniques)

#### **2. Monoclonal:**

None.

#### **3. Peptides:**

None.

### **Phenotype and/or Biological Activity:**

#### **1. Transgenic /**

(pending / analyzed)

other	
<p>Because the lack of a phenotype constitutes a 'negative' result no conclusions can be drawn from this experiment. Further testing will be required to determine any or all of IFN-like proteins' biological activities in vivo.</p>	
2. <i>in vivo</i> assays:	(available, used, proposed)
<p>Not done.</p>	
3. <i>in vitro</i> assays:	(available, used, proposed)
<p>Rat IFN-like Fc fusion protein treatment of several cell lines caused phosphorylation of some cellular proteins (unidentified).</p>	

**References:**

Nothing specifically published on this gene. Lots of references for the interferon family.

**Genomic DNA Sequence (i.e. including all introns and exons):**

The human gene was cloned from genomic DNA. The attached sequence (above) comes from genomic DNA and includes the coding region which is found in one exon, and the flanking regions.

**Ortholog DNA Sequences:**

Human and rat sequences cloned.